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CITRIC ACID #119

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VOLUME 1

GRAS MONOGRAPH SERIES

CITRIC ACID

prepared for
THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

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Summary

Acute Toxicity

The LD₅₀ for citric acid ranges from 5040 mg/kg BW for p.o. administration to mice (2622) to an estimated 337 mg/kg BW for i.v. administration to man (0843). For additional data see Table 6 .

Short-term Studies

Yokotani et al. (2622) fed rats 0.2, 2.4, and 4.8% TAKEDA-citric acid mixed with a commercial diet for six weeks. They observed a decrease in weight, daily food intake, total plasma protein, erythrocyte and leucocyte counts, hematocrit value and hemoglobin content, organ weight (thymus, spleen, prostate and thyroid), and plasma cholesterol. There was an increase in serum GOT activity.

Behnke et al. (0182) gave suckling rats 400 mg/kg citric acid p.o. They found marked differences between the sucklings and adult rats: (a) a delay of acid transport between stomach and small intestine; (b) the acid remained longer in the suckling's intestine; (c) a delayed absorption of citrate in sucklings; and (d) delayed metabolism of citric acid compared with adults. The authors concluded that acidified milk is unsuitable for babies.

Nazario (1652) reported on research suggesting: (a) that citric acid has a destructive effect on dental enamel and (b) that in sensitive children and adults citric acid could impede the circulation of the blood sufficiently to lead to toxicity and death.

Tuft and Ettelson (2436) reported the case of a man who was sensitive to citric acid both naturally present and as an additive in a wide variety of foods.

Ballabriga et al. (0134) studied feeding of premature infants either high or low-protein milk formulas acidified with citric acid for 12 days. They concluded such milk formulas should contain less than 4.5 mEq/kg/day of the acid to prevent development of metabolic acidosis.

Long-term Studies

Bonting and Jensen (0263) fed rats a basal diet containing 1.2% citric acid over their entire life span for three successive generations. The authors noted specifically that no acidosis had been produced. There was no detrimental effect on growth or reproduction. Tissue composition changes observed were: decreased liver Na content; increased muscle Na content; and decreased total P content.

Horn et al. (0994) fed two groups of rats a basal diet containing 3% and 5% citric acid, respectively for two years. A reduction in weight gain and food consumption was observed. There were no observed effects with respect to gross pathology and no significant difference in survival between experimental and control animals.

Special Studies

Cramer et al. (0480) showed that when citrate was incorporated into a vitamin D-free diet containing low P and adequate Ca and was fed to rats, the absorption of Ca was prevented. From observations of negative Ca and P balances, decreased bone ash and increased metaphyseal widths, the authors concluded that citrate had a rachitogenic effect.

Dalderup (0505) found that rats fed a citric acid supplemented non-cariogenic diet, did not develop more caries than controls, but the molars of the animals on the highest dosage levels showed more wear and attrition.

Landauer and Rhodes (1309) found that citric acid (25 mg) significantly reduced embryo mortality and rumplessness when added to insulin treatment at 24 hours. Beaudoin (1309) confirmed these results. He found that it is possible to interfere with the teratogenic action of Trypan Blue in the chicken egg by simultaneous treatment with citric acid.

Biochemical Aspects

Absorption & Distribution

Metabolism & Excretion

Citric acid reportedly is absorbed readily and distributed generally through the tissues; it contributes to biosynthesis of longer-chain fatty acids, especially stearic (0094).

Citric acid enters the Krebs or citric acid cycle (0153); free citric acid in diet may be metabolized somewhat differently from that in foods (2201) but has been recommended as an energy source for patients undergoing surgery (0572). Its metabolism interacts with that of calcium (see Monograph on vitamin D), and patients with calcified stones reportedly retain excessive citrate (2107). Most citrate is excreted in the urine, and in infants, 92% of that passing the glomerulus is reabsorbed (2398).

Effects on Enzymes &

Other Biochemical

Parameters

Citrate has various effects on glycolysis; for example, it inhibits phosphofructokinase (1905). On blood it acts as an anticoagulant (0110). It alters the taste-sense as judged by sensory evaluations (1768), and appears to be a vitamin C antagonist (2626). Other reported actions are summarized in the monograph.

Drug Interactions

Only one report was found; citric acid given with KCl lowered the blood Mg level of cows faster than did KCl alone, but had no effect given by itself (0253).

Consumer Exposure

Citric acid has been used widely in the food industry for more than 100 years as an acidulant, sequestering agent, synergistic antioxidant, dispersing agent, flavor enhancer, and water conditioning agent (0724).

According to the NAS/NRC Comprehensive GRAS Survey, the estimated amount in the average daily diet ranges from 612-1795 mg for infants and children up to 2 years of age to 3125 mg for older children and adults (0678). The estimated maximum amount in the daily diet is 3793 mg and 6792 mg, respectively, for these two groups (0678).

Patents have been granted in the United States for the use of citric acid in instant effervescent beverage powders at levels as high as 56.7% (1573). Other foods in which it is used at high levels are soft drinks, candy, chewing gum, ice cream and ices, and baked goods (0681).

Estimated Acceptable Daily Intakes according to the Joint FAO/WHO Expert Committee on Food Additives are 0-60 mg/kg BW (unconditional) and 60-120 mg/kg BW (conditional), amounts occurring naturally not included (1086).

Domestic production estimates range from 60 to 80 million pounds annually (1389). The total poundage reported to NAS and FEMA in 1972 was 35,898,461 (0680).

CHEMICAL INFORMATION

I. Nomenclature

A. Common name

Citric acid

B. Chemical name

Citric acid (2-Hydroxy-1,2,3,-Propanetricarboxylic acid)

C. Trade names

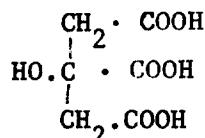
No information.

D. Chemical Abstracts Services Unique Registry Number: 77929

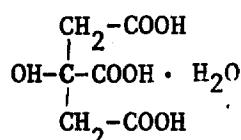
II. Empirical formula

Anhydrous citric acid: $(C_6H_8O \cdot H_2O)$ (the form generally produced (1389).

III. Structural formula



Anhydrous Citric Acid



Citric acid monohydrate

IV. Molecular Weight

Anhydrous citric acid: 192.13

Citric acid monohydrate: 210.14

V. Specifications

A. Commercial grade

Lockwood et al. (1389) give the following specifications for commercial citric acid:

Commercially available citric acid in the United States meets the following USP specifications (21): It contains at least 99.5% of citric acid, calculated on an anhydrous basis. Anhydrous citric acid contains no more than 8.8% water. Ash does not exceed 0.05%. The calcium precipitation test for oxalic acid should be negative. Heavy metals are limited to no more than 10 ppm. Substances readily carbonizable in the presence of hot concentrated H₂SO₄ (90°C for 60 min) must be such that the color formed does not exceed that of 1 part cobaltous chloride solution (59.5 mg CoCl₂.6H₂O in 1 ml 0.289*N* HCl) mixed with 9 parts ferric chloride solution (45 mg FeCl₃.6H₂O in 1 ml 0.289*N* HCl).

Particle-Size Specifications (7). Citric acid, USP, anhydrous, is available in the following U.S. standard mesh sizes:

granular (16-50 mesh)	max 1% on 16-mesh screen max 10% through 50-mesh screen
fine granular (35-100 mesh)	max 1% on 30-mesh screen max 5% on 35-mesh screen max 5% through 100-mesh screen
powder (80-200 mesh)	max 1% on 60-mesh screen min 25% through 200-mesh screen

Citric acid, USP, hydrous, is available in the following mesh sizes:

granular (16-50 mesh)	max 10% on 16-mesh screen max 10% through 50-mesh screen
fine-granular (40-100 mesh)	max 10% on 40-mesh screen max 10% through 100-mesh screen
fine-granular XX (60-200 mesh)	max 25% on 60-mesh screen max 30% through 200-mesh screen

B. Pharmaceutical grade

USP citric acid specifications are presented by the U.S. Pharmacopeia (2445).

Citric Acid



C₆H₈O₇ (anhydrous) 192.13

Citric Acid is anhydrous or contains one molecule of water of hydration. It contains not less than 99.5 percent and not more than 100.5 percent of C₆H₈O₇, calculated on the anhydrous basis.

Description: Colorless, translucent crystals, or white, granular to fine, crystalline powder. Is odorless and has a strongly acid taste. The hydrous form is efflorescent in dry air.

Solubility: Very soluble in water; freely soluble in alcohol; sparingly soluble in ether.

Identification—A solution responds to the tests for *Citrate*, page 892.

Water, page 947: not more than 0.5 percent (anhydrous form) and not more than 8.8 percent (hydrous form), determined by the *Titrimetric Method*.

Residue on ignition, page 901: not more than 0.05 percent.

Oxalate—Neutralize 10 ml. of a solution (1 in 10) with ammonia T.S., add 5 drops of diluted hydrochloric acid, cool, and add 2 ml. of calcium chloride T.S.: no turbidity is produced.

Sulfate—To 10 ml. of a solution (1 in 100) add 1 ml. of barium chloride T.S. to which has been added 1 drop of hydrochloric acid; no turbidity is produced.

Arsenic, page 894: 3 parts per million.

Heavy metals, page 897—Dissolve 2 g. in 10 ml. of water, add 1 drop of phenolphthalein T.S., then add ammonia T.S. until the solution is faintly pink. Add 2 ml. of diluted acetic acid, and dilute with water to 25 ml.; the heavy metals limit is 10 parts per million.

Readily carbonizable substances—Transfer 1.00 g., powdered for the test, to a 22- \times 175-mm. test tube previously rinsed with 10 ml. of sulfuric acid T.S. and allowed to drain for 10 minutes. Add 10 ml. of sulfuric acid T.S., agitate until solution is complete, and immerse in a water bath at $90 \pm 1^\circ$ for 60 ± 0.5 minutes, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube; the color of the acid is not darker than that of a similar volume of Matching Fluid K (see page 921) in a matching tube, the tubes being observed vertically against a white background.

Assay—Place about 3 g. of Citric Acid in a tared flask, and weigh accurately. Dissolve in 40 ml. of water, add phenolphthalein T.S., and titrate with 1 N sodium hydroxide. Each ml. of 1 N sodium hydroxide is equivalent to 64.04 mg. of $C_6H_8O_7$.

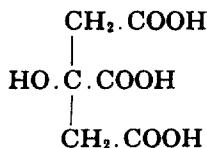
Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate whether it is anhydrous or hydrous.

C. Food grade

Food Chemicals Codex (0456) gives the following specifications for food grade citric acid:

CITRIC ACID



Mol. wt. 192.13

DESCRIPTION

Citric acid is anhydrous or contains one molecule of water of hydration. It occurs as colorless, translucent crystals or as a white, granular to fine crystalline powder. It is odorless, has a strongly acid taste, and the hydrous form is efflorescent in dry air. One gram is soluble in about 0.5 ml. of water, in about 2 ml. of alcohol, and in about 30 ml. of ether. A 1 in 10 solution gives positive tests for *Citrate*, page 926.

SPECIFICATIONS

Assay. Not less than 99.5 percent of $C_6H_8O_7$, calculated on the anhydrous basis.

Water. Anhydrous form, not more than 0.5 percent; hydrous form, not more than 8.8 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).

Oxalate. Passes test.

Readily carbonizable substances. Passes test.

Residue on ignition. Not more than 0.05 percent.

TESTS

Assay. Dissolve about 3 grams, accurately weighed, in 40 ml. of water, add phenolphthalein T.S., and titrate with 1 N sodium hydroxide. Each ml. of 1 N sodium hydroxide is equivalent to 64.04 mg. of C₆H₈O₇.

Water. Determine by the *Karl Fischer Titrimetric Method*, page 977.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Oxalate. Neutralize 10 ml. of a 1 in 10 solution with ammonia T.S., add 5 drops of diluted hydrochloric acid T.S., cool, and add 2 ml. of calcium chloride T.S. No turbidity is produced.

Readily carbonizable substances, page 943. Transfer 1.0 gram, finely powdered, to a 22 × 175-mm. test tube, previously rinsed with 10 ml. of sulfuric acid T.S. and allowed to drain for 10 minutes. Add 10 ml. of sulfuric acid T.S., agitate the tube until solution is complete, and immerse the tube in a water bath at 90 ± 1° for 60 ± 0.5 minutes, keeping the level of the acid below the level of the water during the heating period. Cool the tube in a stream of water, and transfer the acid solution to a color-comparison tube. The color of the acid solution is not darker than that of the same volume of *Matching Fluid K* in a similar matching tube, viewing the tubes vertically against a white background.

Residue on ignition, page 945. Ignite 4 grams as directed in the general method.

Packaging and storage. Store in tight containers.

Labeling. Label to indicate whether it is anhydrous or hydrous.

Functional use in foods. Sequestrant; dispersing agent; acidifier; flavoring agent.

VI. Description

A. General Characteristics

Citric acid is a translucent, crystalline solid. Monohydrate crystals are orthorhombic; those of the anhydrous grade are monoclinic holohedra. Citric acid is odorless and has a pleasant sour (tart) taste (389,2247).

B. Physical Properties

Citric acid is very soluble in water, moderately soluble in alcohol, but only sparingly soluble in ether. Other chemical and physical properties are given in Table 1.

Table 1. Chemical and Physical Properties of Citric Acid (0724)

Chemical Formula	$\begin{array}{c} \text{CH}_3\text{COOH} \\ \\ \text{HOCCOOH} \\ \\ \text{CH}_3\text{COOH} \end{array}$
Molecular Weight	192.12
Physical Aspects	From cold water—colorless, translucent orthorhombic
Melting Point	From hot water—anhydrous, colorless, translucent holohedral class of monoclinic crystals Anhydrous, 153°C; monohydrate softens at 70–75° when heated slowly and melts completely at 135–152°C. With rapid heating the monohydrate liquifies at 100°C
Solubility	
Water	See Figure 1.
Ethanol, 25°C	58.9 g/100 ml
Ether, 25°C	1.84 g/100 ml
Density	Monohydrate, 1.542; anhydrous, 1.665
Molecular Refraction	Monohydrate, 67.11
Refractive Indexes, n_D^{20}	1.493, 1.498, 1.509 (hyd)
Heat of Combustion, ΔH°	Monohydrate, –471.4 kcal/mole Anhydrous, –474.5 kcal/mole Anhydrous, 56.2 lbs/cu ft
Bulk Density	
Ionization Constants	
K_1	8.2×10^{-5}
K_2	1.8×10^{-6}
K_3	3.9×10^{-8}
Heat of Solution, 25°C	–3.9 kcal/mole
Viscosity, 50% Aqueous Solution, 25°C	6.5 cp
Std. Free Energy of Anion Formation (ΔF°_f) , 25°C	–278.8 kcal for aqueous solutions
Buffering Index	2.46
Odor	Odorless
Taste	Tart

C. Stability

Caking occurs at relative humidities above 50–70% and temperatures over 70°F with both USP hydrous and anhydrous grades (1389). The hydrous form is efflorescent in dry air (0456).

Solutions of citric acid are mildly corrosive. Stainless-steel tanks are preferred. Plastic-lined and glass-lined low carbon steel tanks are satisfactory (1389).

Multiwall moisture-proof paper bags and fiber drums are used for packaging hydrous and anhydrous USP citric acid (1389). No ICC shipping label is required (0607).

VII. Analytical Methods

See Table 2.

Table 2. Analytical Methods

Substance(s) Analyzed	Method(s)	Special Reagent(s)	Quantitative Aspects: Amount Determined; Sensitivity; Accuracy	Bibliographic Reference
1. Juices and other similar products	Enzymatic	Lactate dehydrogenase malate dehydrogenase citrate lyase	0.05 mg/ml	0328
2. Fruits & Vegetables	Elution chromatography, paper chromatography, titration	Dowex resin, thymol blue, alcohol and acetate systems for paper chromatography	3.0 mg/100 g fruit or vegetable	1085
3. Urine	Silica-gel chromatography	Silicic acid	0.0095 meq/25 ml	1731
4. In acid medium	Spectrophotometric	Ferrous ammonium sulfate	0.5 to 2.3 mg (<3% error)	2404
5. Fats	Potentiometric titration	Tetrabutylammonium hydroxide	Accuracy \pm 5%	1715
6. In mixtures of organic acids	Fluorimetric	Six enzyme systems	0.1-500 μ g about 2% accuracy	0850
7. In urine, deproteinized plasma and tissue extracts	Fluoroenzymic	Citrate lyase and malic dehydrogenase	Linear sensitivity throughout the range 10^{-5} to 2×10^{-7} M	0472
8. Commercial Wines	Gas-liquid chromatography and formation of trimethylsilyl derivs.	Trimethylchlorosilane and hexamethyldisilazane	10 mg/100 cc sample	1483
9. Tap water and sewage effluents	Anion exchange and gas chromatography	Acetone	1-10,000 parts per billion	0115

VIII. Occurrence and Levels

A. Plants

Citric acid occurs to a certain extent in all living cells which are dependent on carbon compounds for energy (1389). Levels as high as 8% have been reported for unripe lemons. Concentrations in a wide variety of fresh fruits and vegetables are given in Table 3.

B. Animals

Citrate is found in all animal tissues. In man, the normal blood citrate level is about 25 mg/liter (1389).

Zipkin (2679) found citric acid in 180 saliva specimens from 15 male adults in concentrations ranging from 0.44 to 2.40 mg/100 cc (expressed as the monohydrate). The author's results agreed closely with those of previous investigators employing sensitive analytical procedures (2679).

Reinart *et al.* (1929) determined the average citric acid content of bulk milk from an estimated 10,000 cows to be 0.172%. Seventy-two samples collected over a period of one year were analyzed. Average values from eight previous investigations by other workers ranged from 0.08 to 0.23% (references are given in original article).

Hertelendy *et al.* (0957) found mean values for citric acid in liver, kidney, heart, brain, pancreas, and living tissues from laying chickens of 10.8, 14.2, 14.2, 47.2, 66.7, and 50.7 mg/100 grams fresh tissue. Levels up to 400 mg/100 g fresh tissue were found in the isthmus and shell gland of the oviduct. Plasma levels ranged from 2.79 to 8.19 mg/100 ml depending on sex and degree of maturity.

Thunberg (2392), in a review on the occurrence and significance of citric acid in the animal organism, stated that the amount in the human body is approximately 100 grams. Bone contains 0.75% and the soft tissues (including fluids) 15 mg/kg BW. Amounts in other tissues are given in Table 4.

The normal blood serum range is reported as 17-30 micrograms/ml. The total amount of citric acid in the serum of man ("circulating citric acid") is approximately 1 mg/kg BW. Levels for the horse and rabbit are 50 and 100 micrograms/ml, respectively. Human milk contains 0.35-1.25% and the normal daily excretion in the urine is 0.2-1.0 gram (2312).

Table 3. Occurrence and Levels of Citric Acid in Fresh Fruits and Vegetables

<u>Fruit or Vegetable</u>	<u>Level</u>	<u>Reference</u>
<u>Fruit Juice</u>	<u>(Percent)</u>	
Lemon (unripe)	6.0-8.0	1389
Lemon (ripe)	4.0-4.4	0439
Lime (ripe)	0.08	0439
Orange (ripe)	0.6-1.0	0439
Tangerine (ripe)	0.9-1.2	0439
Grapefruit (ripe)	1.2-2.1	0439
<u>Fruit</u>	<u>(mg/100 grams)^a</u>	
Apple, McIntosh	8.1	1085
Strawberry	653-817	1085
Raspberry	1060-1290	1085
Currant, black	1530-3060	1085
Currant, red	733-1355	1085
Gooseberry	982-993	1085
<u>Vegetable</u>	<u>(mg/100 grams)^a</u>	
Tomato (whole)	254	1085
Asparagus, stem - tip (1 inch)	83-228	1085
Cabbage, leaves	49-68	1085
Corn, kernels	21	1085
Eggplant, flesh	10	1085
Lettuce, leaf blade	16	1085
Peas (green)	52	1085
Squash, butternut, flesh-peel	7-25	1085
Turnip, flesh-peel	46-113	1085
Potatoes, stem sect.-bud sect.	116-412	2302
Potatoes	280-510	2099

^a Fresh weight

Table 4. Amounts of Citric Acid in Mammalian Organisms (2392) (With Special Reference to Human Fluids and Tissues)

Organ or Body Fluid	Micrograms/ml Parts per million (approx.)
Whole Blood	15 ^a
Blood Plasma	25
Red Blood Corpuscles	10
Milk	500-1250
Urine	100-750
Semen	2000-4000
Cerebrospinal Fluid	25-50
Mammary Gland	3000
Thyroid Gland	750-900
Kidney and Some Other Organs	20
Liver, Muscle	5
Bone Substance	7500
Amniotic Fluid	17-100
Sweat	1-2
Tears	5-7
Skeletal Muscle, Liver, Kidney, Brain	2-100

C. Synthetics

Table 5. Uses of Citric Acid (1389)

<u>Category</u>	<u>Preparation</u>	<u>Function</u>
Pharmaceuticals	Syrups, solutions, elixirs	Acidulant; flavoring agent
	Effervescent Powders & tablets	To release CO ₂
	Astringents (oral)	Acidulant
	Vaginal jellies & ointments	Spermaticide
Cosmetics	Hair rinses	Lustre agent
	Hair setting fluid	Beauty aid
	Bleaching lotions	Acidifier; sequestrant
Industrial	Metal cleaners	Tarnish & rust remover
	Copper plating	Cleaner; plating aid
	Electropickling	Cleaner; stabilizer
	Tanning	Acidifier
	Bottle washing compounds	Prevents hard-water scale
	Decontaminants	Removal of radioisotopes
	Mordant	Brighten colors
	Special inks	

Citric acid is used also in the treatment of rickets and to dissolve urinary bladder calculi (2247).

Faith et al. (0647) gave the following use pattern for citric acid:

Beverages	60%
Pharmaceuticals	18%
Sodium Salts & Esters	10%
Industrial	10%
Cosmetics	2%

See BIOCHEMICAL ASPECTS, VI. Consumer Exposure, for occurrence in foods.

BIOLOGICAL DATA

I. Acute Toxicity

A. Mice

1. In 1948 Gruber and Halbeisen (843) determined the LD₅₀ for rapid i.v. injection to mice (average weight, 24 g), at a constant rate of 6 cc/minute until death, of a 0.25 M solution of citric acid to be 42 mg/kg. The LD₅₀ for i.p. injection to mice (average weight 19 g) of 0.477 M citric acid was 961 mg/kg.

The observed train of symptoms which the authors suggested were identical with those of calcium ion deficiency were: increased general activity, hypernea, vaso-dilatation of the peripheral vessels, salivation, muscle twitching, clonic and tonic convulsions, cyanosis and Cheyne-Stokes respirations. The authors concluded from a comparison with the LD₅₀ of citrate salts that the acid part of the molecule rather than the citrate is responsible for its toxicity.

2. In 1958 Horn *et al.* (994) produced immediate convulsive death in mice when a 2% solution of citric acid (pH 2.50; LD₅₀ = 203 mg/kg) was delivered i.v. at the rate of 0.01 ml per second. No gross pathology other than hemorrhagic lungs was seen at autopsy.

3. In 1971 Yokotani *et al.* (2622) of the Takeda Research Laboratory compared the acute toxicities of commercial citric acid (CA) and TAKEDA-citric acid (NPC, a refined final product of citric acid, produced by yeast fermentation). The LD₅₀ and behavioral signs after administration were found to be similar in mice (ICR-JCL, 4-week-old males 20-24 g).

(a) P.O. LD₅₀ of NPC, 5790 and of CA 5040 mg/kg/BW. Activation of spontaneous movements followed in an hour by motor ataxia, mydriasis and decreased respiration rate and heartbeat. Death within 20 to 180 minutes. Hemorrhage of the gastric mucosa was seen at autopsy.

(b) I.P. LD₅₀ of NPC 940 and of CA 960 mg/kg BW. Immediate stretching and slow crawling with death by respiratory or cardiac failure. The surface of the spleen and liver were found to be covered with a thin whitish membrane at autopsy, and the liver was slightly hypertrophied.

(c) S.C. LD₅₀ of NPC 2700 mg/kg BW. Behavioral signs similar to p.o. and i.p. routes. Death occurred from respiratory failure or emaciation. Inflammation and necrosis were seen at the inspection site.

Table 6. Citric Acid Acute Toxicity Table
(by substance or constituent thereof)

Substance	Animal (Species)	Sex & No. (M or F)	Route (p.o., i.v., s.c., i.p., i.m., other)	Dosage (mg/kg BW)	Measurement (LD ₅₀ , ED ₅₀ or other)	Reference
Citric acid	White mice	ca. 70	Rapid i.v.	42	LD ₅₀	Gruber & Halbeisen (0843)
Citric acid	White mice	ca. 120	i.p.	961	LD ₅₀	Ibid
Citric acid	Albino mice	M 13	i.v.	203	LD ₅₀	Horn <u>et al.</u> (0994)
Citric acid	Mice	M 6	p.o.	5040 (4520- 5665)	LD ₅₀	Yokotani <u>et al.</u> 2622
Citric acid	Mice	M 6	i.p.	960 (888-1037)	LD ₅₀	Ibid
		M 6	p.o.	5790 (4990- 6720)	LD ₅₀	Ibid
TAKEDA-citric acid	Mice	M 6	i.p.	940 (872-1019)	LD ₅₀	Ibid
TAKEDA-citric acid	Mice	M 6	s.c.	2700 (2390- 3050)	LD ₅₀	Ibid
Citric acid	Albino rats	ca. 75	i.p.	884	LD ₅₀	Gruber & Halbeisen (0843)
TAKEDA-citric acid	Rats	M 6	p.o.	11,700 (19,080- 13,570)	LD ₅₀	Yokotani <u>et al.</u> (2622)
TAKEDA-citric acid	Rats	M 6	i.p.	725 (690-762)	LD ₅₀	Ibid
TAKEDA-citric acid	Rats	M 6	s.c.	2700 (2390- 3050)	LD ₅₀	Ibid
Citric acid	Rabbits	ca. 25	i.v.(on 358 mm/min)	ca. 330	LD ₅₀	Gruber & Halbeisen (0843)

B. Rats

1. In 1948 Gruber and Halbeisen (0843) determined the LD₅₀ for i.p. injection of 0.381 M citric acid to rats (average weight 120 g) to be 884 mg/kg. The visible responses were similar to those seen with mice (Section A1 above).

2. In 1971 Yokotani et al. (2622) determined the LD₅₀ for TAKEDA-citric acid administered to male rats (SD-JCL, 5-week old male, 110 to 140 g); p.o. 11,700 mg/kg; i.p. 725 mg/kg and s.c. 5500 mg/kg. A few minutes after administration the animals showed mydriasis and a decrease in respiration rate. Death was due to respiration failure or emaciation. The same behavioral and autopsy observations were made with the rats as with the mice as described in Section A3 above.

C. Rabbits

1. In 1948 Gruber and Halbeisen (0843) injected rabbits (1.75 to 2.75 kg) into the lateral ear veins with 0.477 M citric acid at a constant rate of 0.75 cc/minute. The LD₅₀ was found to be ca. 330 mg/kg. The visible responses were found to be similar to that observed with mice and rats (see Sections A1 and B1 above).

D. Dogs

1. Gruber and Halbeisen (0843) also injected three dogs (10 to 15 kg) having lumbar cordotomies with 2.1 g/kg/100 cc citric acid i.v. at a constant rate of 0.67 cc/minute until death occurred. There was a gradual fall in blood pressure during citric acid injection until near death when it rapidly dropped to zero.

E. Man

1. Gruber and Halbeisen (0843) estimated the LD₅₀ for i.v. administration to man to be about 337 mg/kg which is near that for the slow i.v. of rabbits (see above Section C).

II. Short-term Studies

A. Rats

1. In 1971 Yokotani et al. (2622) of the Takeda Research Laboratories fed rats high oral doses of TAKEDA-citric acid (NPC), a refined final product of citric acid produced by yeast fermentation for six weeks. Four groups of 10 rats each (male SD-JCL, 98 to 112 g, 29 to 35 days old) were fed a commercial diet to which 0, 0.2, 2.4, and 4.8% NPC were added respectively.

The results of studies on growth, blood, urine, biochemistry and morphology are summarized in Figure 1, Table 7, Table 8, and Table 9. It was observed that:

- (a) The weight gain of all three experimental groups decreased after the first week (see Figure 1).
- (b) The daily food intake of the treated animals was reduced compared to the controls.
- (c) There was a significant decrease in total plasma protein in the treated animals.
- (d) At the higher doses there was a small decrease in erythrocyte and leukocyte counts, hematocrit value and hemoglobin content (see Table 7).
- (e) At the highest dose there was a decrease in plasma cholesterol level and an increase in serum GOT activity (see Table 8).
- (f) Treated animals had a decreased organ weight (see Table 9) especially at the highest dose level in thymus, spleen, prostate and thyroid.
- (g) The inner surface of the right hepatic lobe of treated animals showed yellowish-white biliary tubercles.
- (h) At the highest dose level histological examination showed a slight atrophy of the cortex of the thymus and of the lymph follicle of the spleen.

2. In 1970 de Albuquerque and Henriques (0156) fed rats a diet supplemented with citric acid. The weight changes in six rats fed a diet supplemented with 2.5% citric acid for nine days are shown in Table 10. It can be seen that during the first few days there was either a decrease or no change in weight. After a week, their growth tended to become more like that of animals on a normal diet (see Table 11).

B. Rabbits

1. In 1958 Arai and Ishigami (0093) investigated whether as Arai and coworkers (references in original article) had previously found with two other Krebs cycle acids, fumaric and pyruvic, the prolonged administration of citrate would produce arteriosclerosis in rabbits. The experimental procedure and degree of arteriosclerosis produced is shown in Table 12. The rabbits (5 m and 4 f, 1350 to 2400 g) were fed a diet of bean-curd residuum and injected (intra-abdominal) with 2.0 cc of a 3% sodium citrate solution three times weekly for from five to 25 weeks.

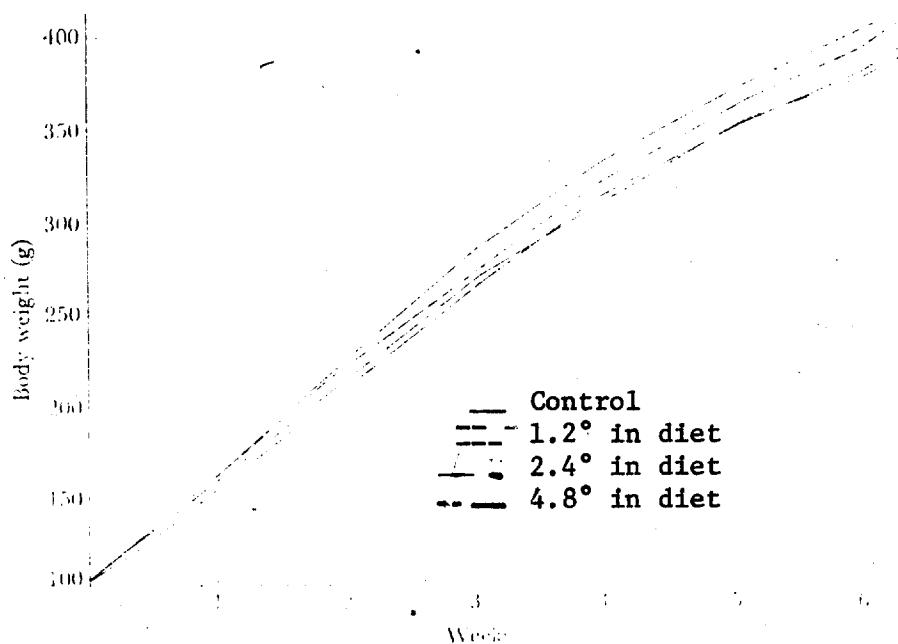


Figure 1. Mean Growth Curves in Male Rats Treated Orally with NPC for 6 Weeks (2622)

Table 7. Blood Picture in Male Rats Treated Orally with NPC for 6 Weeks (2622)

Dosage % in diet	No. of rats	Erythrocytes $(\times 10^6/\text{mm}^3)$	Hemato- crit (%)	Hemo- globin (g/dl)	Leucocytes $(\times 10^3/\text{mm}^3)$	Differentials				
						Neutro- phils	Lympho- cytes	Mono- cytes	Eosino- philis	Beso- phils
0	5	763.0 ^a +19.2	46.6 +0.9	16.1 +0.5	6.42 +1.29	6.3	90.4	2.6	0.2	0
1.2	5	766.0 +27.0	45.4 +1.5	16.1 +0.8	8.20 +1.09	3.4	93.3	2.6	0.2	0
2.4	5	738.0 +61.4	45.0 +3.5	15.8 +0.8	8.02 +1.12	10.1	85.1	3.6	0.6	0
4.8	5	730.0 +60.0	44.2 +3.8	15.3 +1.5	7.38 +1.97	10.3	85.3	2.3	0.6	0

a: Mean \pm S.D.

Table 8. Results of Biochemical Examination Male Rats Treated Orally with NPC for 6 Weeks (2622)

Dosage % in diet	No. of rats	T. prot. (g ^{0.1})	Alb. (g%)	Glob. (g%)	A/G ratio	Ca ⁺⁺ (mg%)	T. chol. (mg%)	Glu. (mg%)	BUN (mg%)	Creat. (mg%)	T. bili. (mg%)	Alk. phos. (mU/ml)	LDH (mU/ml)	GOT (mU/ml)	GPT (R.F.U./ ml)	BSP retention (mg%)
0	5	6.51 ^a ± 0.17	3.01 ± 0.33	3.49 ± 0.38	0.88 ± 0.26	10.05 ± 0.31	77.8 ± 6.0	120.4 ± 14.9	13.9 ± 2.4	0.53 ± 0.06	0.12 ± 0.04	153.0 ± 33.3	113.4 ± 13.5	143.4 ± 22.3	39.4 ± 3.3	0.32 ^{(3)c} ± 0.05
1.2*	5	6.26 ± 0.18	2.62 ± 0.06	3.64 ± 0.16	0.72 ± 0.03	9.65 ± 0.51	75.0 ± 10.8	117.8 ± 8.2	15.4 ± 1.3	0.55 ± 0.03	0.10 ± 0.01	183.6 ± 16.5	119.0 ± 17.3	162.0 ± 12.7	40.6 ± 4.9	0.34 ⁽³⁾ ± 0.09
2.4	5	6.16 ^{b*} ± 0.22	2.86 ± 0.32	3.29 ± 0.40	0.89 ± 0.23	9.62 ± 0.35	75.4 ± 6.2	140.4 ± 15.0	13.5 ± 1.6	0.53 ± 0.06	0.10 ± 0	176.4 ± 22.2	113.0 ± 14.4	164.4 ± 11.2	43.0 ± 2.7	0.41 ⁽³⁾ ± 0.08
4.8	5	6.22 ± 0.32	2.80 ^d ± 0.53	3.42 ± 0.51	0.85 ^d ± 0.29	9.69 ± 0.45	70.2* ± 1.8	125.2 ± 17.7	12.9 ± 1.2	0.59 ± 0.07	0.10 ± 0.01	166.4 ± 4.9	137.6 ± 37.1	175.0* ± 16.2	44.0 ± 4.2	0.38 ⁽³⁾ ± 0.11

a: Mean ± S.D.

b: Significantly different from control, *P<0.05.

c: No. of rats used.

d: One of animals: 1.95 g% (Alb.) and 0.49 (A/G ratio).

Table 9. Mean Organ Wet Weights in Male Rats Treated Orally with NPC for 6 Weeks (2622)

Absolute Dosage % in diet	No. of rats	Final B. wt. (g)	Carcass		Brain (g)	Heart (g)	Lungs (g)	Liver (g)			
			(g)	(%)							
Absolute	0	409.0 ^a ± 19.2	312.9 ± 18.9	76.8 ± 1.7	2.14 ± 0.08	1.29 ± 0.11	1.38 ± 0.09	12.24 ± 2.37			
	1.2	405.4 ± 14.0	309.5 ± 15.0	76.3 ± 2.1	2.14 ± 0.09	1.21 ± 0.09	1.38 ± 0.07	12.13 ± 2.27			
	2.4	391.2 ± 23.4	297.2 ± 18.4	76.0 ± 1.7	2.07 ± 0.07	1.25 ± 0.22	1.39 ± 0.15	12.36 ± 3.38			
	4.8	390.5 ± 23.1	295.5 ± 21.5	75.6 ± 2.5	2.03* ± 0.10	1.23 ± 0.09	1.39 ± 0.15	11.70 ± 2.43			
	0	100	76.8		0.52	0.31	0.34	2.99			
Relative	1.2	8	100	76.3		0.53	0.30	0.34			
	2.4	8	100	76.0		0.53	0.32	0.35			
	4.8	8	100	75.6		0.52	0.31	0.35			
	0	8						3.00			
Absolute Dosage % in diet	Kidneys	R	L	Spleen	R	Testes	Prostate	Ad- renal glands	Thy- roid (mg)	Hypo- physis (mg)	Thymus (g)
		1.43 ± 0.12	1.50 ± 0.14	0.76 ± 0.11	1.49 ± 0.14	1.49 ± 0.12	0.63 ± 0.12	56.1 ± 9.3	16.6 ± 3.2	13.3 ± 1.7	0.68 ± 0.11
		1.46 ± 0.18	1.43 ± 0.14	0.75 ± 0.11	1.57 ± 0.62	1.40 ± 0.22	0.85* ^b ± 0.22	55.4 ± 8.2	16.5 ± 3.6	13.4 ± 1.3	0.63 ± 0.08
		1.40 ± 0.17	1.40 ^(e) ± 0.18	0.71 ± 0.10	1.53 ± 0.06	1.53 ^(e) ± 0.07	0.60 ± 0.16	54.6 ± 9.8	15.6 ^(e) ± 2.6	12.7 ± 1.5	0.68 ± 0.19
	R	1.45 ± 0.20	1.51 ^(e) ± 0.19	0.68 ± 0.14	1.45 ± 0.36	1.57 ^(e) ± 0.07	0.65 ± 0.18	54.4 ± 9.8	17.1 ^(e) ± 1.9	12.7 ± 1.7	0.53* ± 0.10
		0.36 ± 0.20	0.37 ± 0.19	0.18 ± 0.14	0.36 ± 0.36	0.36 ± 0.07	0.15 ± 0.18	13.7 ± 9.8	4.0 ± 1.9	3.2 ± 1.9	0.17 ± 0.10
		0.36 ± 0.20	0.35 ^(e) ± 0.19	0.18 ± 0.14	0.39 ± 0.36	0.34 ± 0.07	0.21 ± 0.18	13.7 ± 9.8	4.1 ± 1.9	3.3 ± 1.7	0.15 ± 0.10
		0.36 ± 0.20	0.35 ^(e) ± 0.19	0.18 ± 0.14	0.39 ± 0.36	0.39 ^(e) ± 0.07	0.15 ± 0.18	13.9 ± 9.8	3.9 ^(e) ± 1.9	3.2 ± 1.7	0.17 ± 0.10

R: Right, L: Left.

a: Mean ± S.D.

b: Significantly different from control, *P<0.05.

c: No. of rats used.

Table 10. Weight Changes of Rats Fed a Diet Containing 2.5% Citric Acid (0156)

Weight g	Rat					
	1	2	3	4	5	6
Initial	75	80	70	80	60	70
After 3 days	65	72	60	75	55	70
After 6 days	70	80	60	75	55	72
After 9 days	80	90	70	85	65	86

Table 11. Weight Changes of Rats Fed a Normal Diet (0156)

Weight g	Rat					
	1	2	3	4	5	6
Initial	72	70	80	65	70	65
After 3 days	90	85	87	88	100	83
After 6 days	100	100	104	100	117	100

It was found that with prolonged treatment arteriosclerotic lesions of the aorta with relatively marked calcification in the media were produced. With shorter treatment there was edematous degeneration under the intima to the media. No lipid was found in any of the rabbits.

The authors considered the etiology to be an induced metabolic disturbance of polysaccharide in the arterial wall induced in part by the excess dose of citrate. The severe calcification seen was attributed to the deposition of the Ca from the bloodstream which was increased by the action of citrate.

The deposition and binding of mucoid substance to elastic fibers was considered to be the necessary condition preceding calcification. The authors concluded that their observations could shed light on the cause of arteriosclerosis in man.

Table 12. Experimental Procedure and Degree of Arteriosclerosis in Rabbits (0093)

Animal No.	Sex	Body weight (g)	Treatment	Period (wks.)	Degree of Arteriosclerosis
CA 5	m.	1900		5	-
CA 3	m.	1800		10	-
CA 8	f.	2300	3.0% sodium citrate solution	10	+
CA 7	m.	1350	sodium citrate solution	13	++
CA 6	f.	2100	2.0cc	15	+
CA 9	m.	1500	thrice a week	19	++
CA 2	f.	2000	intraabdominal inj.	20	-
CA 4	f.	1350		22	++
CA 1	m.	2400		25	++

C. Humans

1. In 1952, Nazario (1652) reviewed the literature bearing on the use of citric acid in foods and cited the following reports (all references given in the original paper):

- (a) The destructive effect of citrate on dental enamel with resultant caries;
 - (1) Shear et al. in 1929 observed that citrate forms a soluble ionized compound with calcium.
 - (2) Clure and Ruzicka in 1946 observed that citrate in an almost neutral drink had a pronounced destructive effect on the dental enamel in vivo.
 - (3) Zipkin in 1947 suggested a relation between salivary citrate and erosion of enamel.
 - (4) Newman in 1948 found no tooth destruction from the consumption of fruits high in citric acid.

(b) Leschke in 1932 reported that citric acid could impede the circulation of the blood sufficiently to lead to toxicity and death, which resembled a similar effect of oxalic acid. This effect in sensitive children can cause circulatory deficiencies and unconsciousness. A young woman who ingested a laxative dose of 25 g citric acid, vomited continuously almost to the point of death.

The author concluded that a 70 kg adult could tolerate 53 g of citric acid daily without damage to health.

2. In 1956, Tuft and Ettelson (2436) described the case of a 37-year old man who was allergic to weak organic acids and particularly to citric acid. After consumption of foods containing citric acid, e.g. grapes (or anything made from grapes), chocolates and certain kinds of candies and carbonated beverages, the patient developed symptoms such as canker sores and headaches, frequent spells of general lassitude, vague pains, irritability and inability to concentrate. Intracutaneous skin tests with chocolate and other foods were negative. Direct application of citric acid crystals to the oral mucosa reproduced the ulcer in the patient but not in the controls. Similar positive results were seen with mucous membrane contact tests with citric acid containing substances.

Avoiding foods containing citric acid was followed by marked relief both of the ulcers and of the other general symptoms which had been medically regarded as functional. The authors concluded that this demonstrated that persons could be allergic to certain substances present in foods such as weak organic acids, despite having a negative skin test to these foods (commercial chocolate bars contain citric acid).

3. In 1969, Ballabriga et al. (0134) examined the metabolic and clinical responses of prematures fed various milk formulas with varying protein contents and acidified with a large amount of citric acid. The 12-day study was carried out with 43 clinically "normal" 12-day old premature infants with a calorie intake of 120 cal/kg BW/day.

A high protein diet (3.19 g proteins/100 ml) with a large amount of citric acid (10.62 mEq/kg/day or 0.5 g/100 ml) induced biochemical metabolic acidosis without clinical signs. The same amount of citric acid with a low protein diet (1.62 g proteins/100 ml) did not.

The authors concluded that milk formulas acidified with citric acid when used in premature feeding should contain less than 4.5 mEq/kg/day of the acid.

III. Long-term Studies

Rats

1. In 1952, Bonting (0264) wrote a doctoral thesis on his study of the effect of prolonged feeding of citric acid on the growth, reproduction, mortality rate, hematology, histopathology and teeth of rats. Citric acid was added to the basal diet of the experimental groups of albino Wistar rats, as shown in Table 13. Two successive generations received a diet with up to 1.2% citric acid for up to 12 months.

The observations summarized were:

- (a) No effect on growth.
- (b) No effect on reproduction.
- (c) No effect on mortality rate.
- (d) No effect on blood picture.
- (e) An increased dental attrition without signs of caries.
- (f) No acidosis was produced.
- (g) No evidence of demineralization.
- (h) No decrease in N retention nor increase in the sum of the urinary ammonia and urea excretion.
- (i) Less than 3% of the ingested citric acid was excreted in the urine.
- (j) A significant decrease in the ash content and an increase in the calcium content of the tibia. In some cases a similar effect was noted in muscle.

Table 13. Dietary Groups (0264)

Group	Acid Added	Av. Intake in mg/d/kg	For Adult Rat in meq/d/kg ¹)
PE	Citric acid	0.15%	1.0
PF	Citric acid	0.45%	3.1
PG	Citric acid	1.20%	8.3

1) at pH 7.4 citric has 2.0 meq/mM bound alkali

The author concluded that consumption of beverages acidified with citric acid would not be harmful.

2. In 1956 Bonting and Jensen (0263) continued the research into the effect of prolonged ingestion of citric acid. The experiments were carried out with three successive generations and over the entire life span of albino rats. A basal diet designed to resemble the average Dutch diet plus 1.2% citric acid represented the maximum daily intake possible based on body weight, if the total daily caloric requirements were obtained daily from the sugar content of soft drinks. Studies were made of growth, reproduction, hematology, gross and microscopic organ appearance, mineral and nitrogen metabolism and tissue composition.

The authors concluded that their experiments had shown:

- (a) No acidosis produced.
- (b) No loss of Ca and other "fixed bases".
- (c) No loss of N.
- (d) No change in the mineral composition of the blood serum.
- (e) Tissue composition changes noted were: decreased Na content of the liver; decreased total P content; increased Na content of the muscle.
- (f) No harmful effect on the growth of three successive generations on the citric acid diet.
- (g) No harmful effect on the reproduction of animals on the citric acid diet for 29 and 40 weeks.

3. In 1957 Horn et al. (0994) conducted a two-year chronic feeding study with citric acid. A basal diet containing 3% citric acid or 5% citric acid was fed to two groups of 20 male albino rats for two years (Carworth Farms strain, 60 g) respectively with 20 males on the basal diet alone as controls. At the end of the two-year period, survivors were sacrificed. The results are summarized in Tables 14, 15, and 16.

Table 14. Summary of Average Body Weights of Albino Rats (0994)

(Controls received the basal diet. Other animals received the basal diet containing the indicated percentage of the citric acid.)

<u>Week</u>	<u>Control</u>	<u>Average Body Weight in Grams</u>	
		<u>Males</u>	
		<u>Citric Acid</u>	
0	59	62	61
8	269	239	225
16	325	298	278
24	361	329	320
32	377	328	339
40	397	370	361
48	423	393	377
56	428	400	388
64	426	407	401
72	407	400	389
80	408	411	391
88	413	411	389
96	432	409	393
104	440	417	397

Table 15. Summary of Data for Albino Rats Receiving Basal Laboratory Diet or Basal Diet with Citric Acid for 2 Years (0994)

(Percent of survival based on length of survival as well as number of animals)

Level	Sex	No. of Rats	Av. Body Weight g		Food Consumed g	Compound Consumed mg	Survival %
			Start	Finish			
Control	M	20	8	59	440	16.8	82.5
Citric acid							
3%	M	20	14	62	417	17.1	512
5%	M	20	16	61	397	15.7	784
							95.0

Table 16. Autopsy Data for Male Rats Fed Citric Acid for 2 Years (0994)

Male Group	Deaths			Sacrificed		
	Lung Pathology	Tumors	Other Causes	Total Deaths	Lung Pathology	Tumors
Control	7	3	3	12	4	1
Citric						
3%	1	2	3	6	1	1
5%	1	2	1	4	4	1

The results showed that:

- (1) During the rapid growth period the weight gains of both the citric acid groups were significantly less than the controls.
- (2) There was a slight but consistent reduction in food consumption by the 5% group.
- (3) There was no evidence of gross pathology in the experimental animals.
- (4) There was no significant difference in survival as compared to controls.

IV. Special Studies

A. Mice

1. In 1955, Dubos (0574) studied the susceptibility of mice to experimental tuberculosis. The experiments were carried out with four virulent and six attenuated strains of tubercle bacilli of human or bovine origin using albino mice bred at the Rockefeller Institute (29 to 32 days old, 15 to 18 g).

The results as summarized in Tables 17, 18, 19 and Figures 2, 3, and 4 show that adding citric acid to any of the test diets shortened the life expectancy of tuberculous mice.

B. Rats

1. In 1956, Cramer *et al.* (0480) investigated the effect of incorporating citrates into a vitamin D-free diet containing low P and adequate Ca when

Table 17. Effect of Citrate in Diets Containing 4 to 5 Percent Skim Milk (see Figure 2) (0574)

Fig. No.	Percentage composition of diets*						Weight gain gm.	Cumulative number of mice (out of 10) dead at				
	Skim milk	Wheat flour	Cerelose	Peanut oil	Cocoa butter	Citrate		3 wks.	5 wks.	7 wks.	9 wks.	11 wks.
3 A-a	4	45	50	—	—	—	3.0	1	7	10	—	—
3 A-ac	4	45	40	—	—	10	-0.5	3	8	9	10	—
3 A-b	4	45	30	20	—	—	3.8	0	1	2	3	—†
3 A-bc	4	45	20	20	—	10	0.4	0	9	10	—	—
3 A-c	4	45	30	—	20	—	3.1	0	4	5	6	—†
3 A-cc	4	45	20	—	20	10	0	3	10	—	—	—
3 A-d	5	74	20	—	—	—	8.2	0	0	3	7	10
3 A-dc	5	66	20	—	—	8	2.5	0	0	6	10	—
3 A-e	5	74	—	20	—	—	11.0	0	0	1	7	8
3 A-cc	5	66	—	20	—	8	6.7	0	0	3	10	—
3 A-i	5	74	—	—	20	—	8.9	0	0	5	9	10
3-A-ic	5	66	—	—	20	8	5.8	0	1	9	10	—
3 A-g	5	74	—	—	20	—	Not weighed	0	3	9	10	—
3 A-gc	5	66	—	—	20	8	Not weighed	3	7	9	10	—
3 A-h	5	74	—	20	—	—	Not weighed	1	2	8	10	—
3-A-hc	5	66	—	20	—	8	Not weighed	2	6	9	10	—

* 1 per cent salt added.

† — experiment discontinued.

Table 18. Effect of Citrate in Diets Containing 15 to 19 Percent Skim Milk (see Figure 3) (0574)

Fig. No.	Percentage composition of diets*							Weight gain gm.	Cumulative number of mice (out of 10) dead at				
	Skim milk	Wheat flour	Cerelose	Peanut oil	Cocoa butter	Lard	Citrate		3 wks.	5 wks.	7 wks.	9 wks.	11 wks.
3 B-i	15	64	20	—	—	—	—	10.8	0	0	2	7	8
3 B-ic	15	56	20	—	—	—	8	6.1	1	1	4	10	—
3 B-j	15	64	—	20	—	—	—	10.1	0	0	1	4	6
3 B-jc	15	56	—	20	—	—	8	7.3	0	1	3	8	9
3 B-k	15	64	—	—	20	—	—	10.5	0	0	2	4	6
3 B-kc	15	56	—	—	20	—	8	5.3	0	1	2	6	8
3 B-l	19	50	30	—	—	—	—	Not weighed	0	0	2	5	9
3 B-lc	19	50	20	—	—	—	10	Not weighed	1	3	5	8	10
3 B-m	19	50	10	—	20	—	—	Not weighed	0	0	4	7	9
3 B-mc	19	50	—	—	20	—	10	Not weighed	0	1	6	10	—
3 B-n	19	50	10	—	—	20	—	Not weighed	0	0	2	8	10
3 B-nc	19	50	—	—	—	20	10	Not weighed	0	2	6	10	—

* 1 per cent salt added.

Table 19. Effect on Mouse Tuberculosis of Citrate, Glutarate, or Alcohol Added to Drinking Fluid (0574)

Fig. No.	Diet	Drinking fluid	Cumulative number of mice (out of 10) dead at			
			3 wks.	4 wks.	5 wks.	6 wks.
4-a	Pellets <i>ad lib.</i>	0.8 per cent NaCl	0	1	5	10
4-b	" " "	2 per cent Na citrate (pH 6.0)	2	7	9	10
4-c	" " "	1 per cent Na glutarate (pH 6.0)	1	7	9	10
4-d	" " "	5 per cent alcohol in saline	0	2	7	9

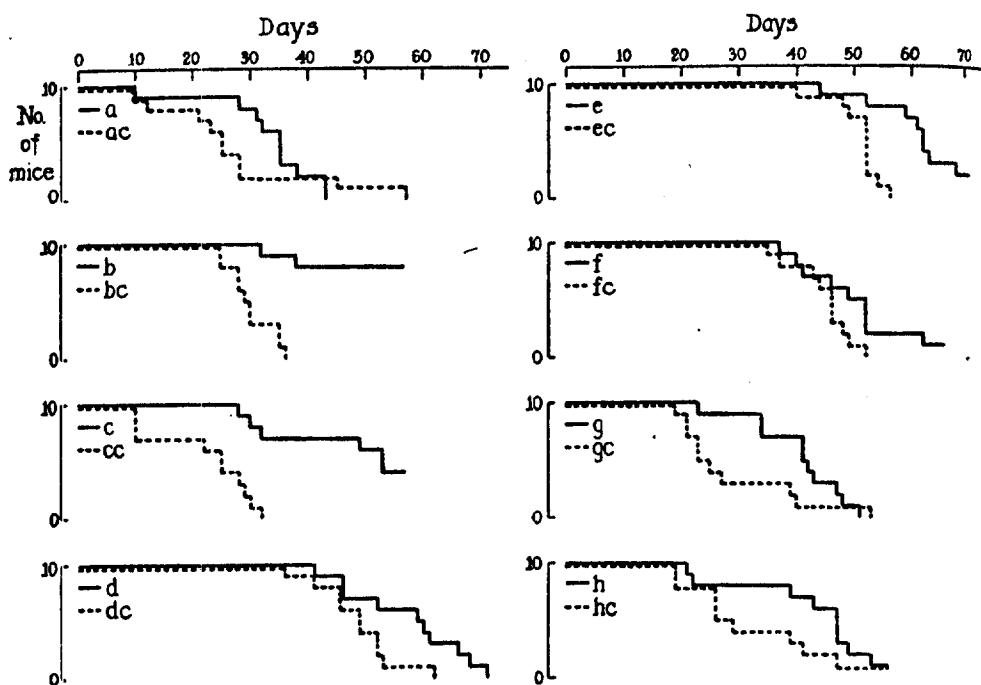


Figure 2. Effect of citrate on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table 17. (0574).

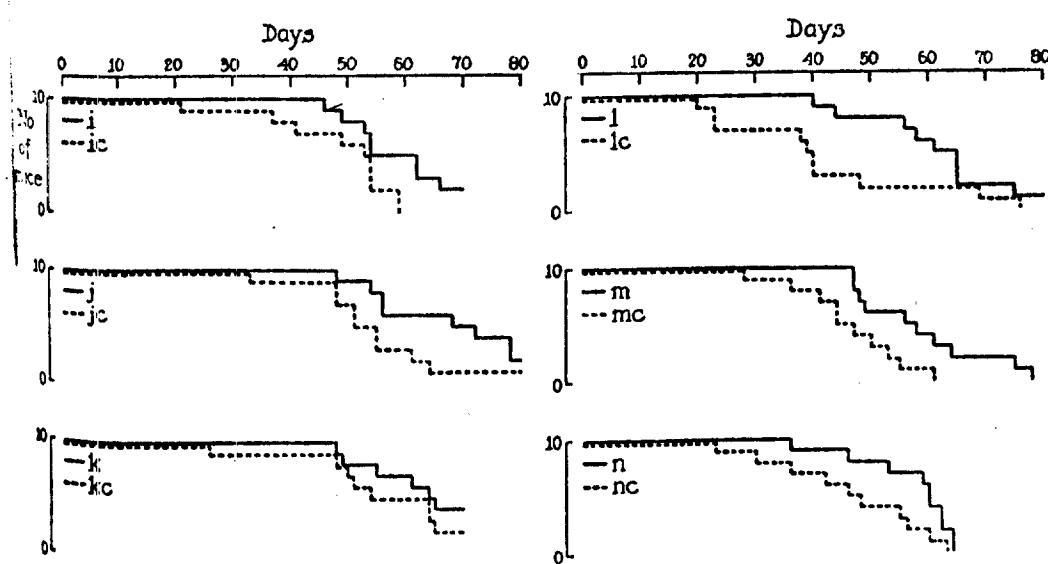


Figure 3. Effect of citrate on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table 18. (0574)

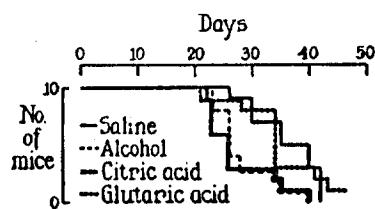


Figure 4. Effect of citrate, glutarate, or alcohol on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table 19. The symbols for glutaric acid and alcohol have been inadvertently reversed in Figure 4 (the data in Table 19 are correct). (0574)

fed to rats. The experimental protocol and effect on Ca absorption for diets with and without citric acid and vitamin D fed to groups of six or seven rats (Sprague-Dawley, 76 to 80 g) is summarized in Table 20.

The results show that citrate greatly increased the fecal excretion of Ca when Ca intake was almost constant. When D was not given, citrate completely prevented the absorption of Ca.

From the observed negative Ca and P balances (see Table 21) decreases in bone ash as calculated on a dry weight or percentage basis and increases in average metaphyseal widths (see Table 22), the authors concluded that citrate had a rachitogenic effect.

2. In 1959 Dalderup (0505) studied dental caries in albino rats receiving citric acid in a dry non-cariogenic diet. Three experimental groups were used (with controls for all three groups).

Group I; 68 rats, 6 weeks to 7 months old fed diet with either 1.5, 4.5, or 2.0 g citric acid per kg food.

Group II; 19 rats, 3 and 10 months old fed the same three supplemented diets as Group I.

Group III; 6 rats, two months old fed 312 mg citric acid per 10 g basal diet. (These animals were one-year old at time of caries evaluation.)

It was found that the molars of animals on the highest dosages showed results with enamel scores in the ten-month old significantly lower than in the three-month old group. There was no difference in caries development in experimental and control animals.

C. Chicks

1. In 1952 Landauer and Rhodes (1309) investigated whether citric acid amongst other agents would be effective in preventing the teratogenic consequences of insulin injection. They found that citric acid greatly reduced embryo mortality and incidence of rumplessness when added to insulin treatments at 24 hours (see Table 23). The results were highly significant in both respects (chi square χ^2) for embryo mortality = 46.035, P = 0.001; for incidence of rumplessness χ^2 = 10.165, P = 0.0005).

Table 20. Effect of Citric Acid and Vitamin D on Ca Absorption (0480)

The ration fed contained 0.5% Ca, as calcium carbonate, and 0.015-0.017% P. Citric acid was added as 0.02 mole Na citrate plus 0.02 mole citric acid per 100 g of ration. The values given in the table are averages from seven rats with the exception of those for the group which received neither citric acid nor vitamin D. In this group the values from six rats were averaged.

Period days	Ca intake		Net Ca absorbed	
	With vitamin D mg.	Without vitamin D mg.	With vitamin D mg.	Without vitamin D mg.
Without citric acid				
4th-6th	98.7	105.8	51.7	33.5
7th-9th	98.9	105.2	57.5	27.3
10th-12th	105.4	106.8	56.3	18.2
Total:	303.0	317.8	165.5	79.0
9 days	(289.9-320.4)	(302.3-320.4)	(131.0-200.1)	(64.9-90.0)
With citric acid				
4th-6th	103.2	106.5	27.1	-0.5
7th-9th	105.6	107.5	44.5	-3.6
10th-12th	105.3	107.2	48.1	15.6
Total:	314.1	321.2	119.7	11.5
9 days	(299.9-322.5)	(316.6-322.5)	(103.4-171.8)	(-23.2)-(+29.7)

Table 21. Effect of Citric Acid and Vitamin D on Ca and P Balances (0480)

The values given in the table are average values from 6 to 7 rats in each group.

Period days	Urinary Ca		Fecal Ca		Ca Balance		P Balance	
	With vitamin D mg.	Without vitamin D mg.						
Without citric acid								
4th-6th	55.6	62.0	46.9	72.8	-3.8	-28.6	-3.1	-6.9
7th-9th	55.5	55.7	41.4	77.8	+2.0	-28.4	-1.8	-5.4
10th-12th	64.9	56.5	49.1	88.5	-8.6	-38.3	-2.0	-5.8
Total:	176.0	174.2	137.4	239.1	-10.4	-95.3	-6.9	-18.1
9 days	(140.0- 215.5)	(161.8- 187.5)	(89.8- 181.0)	(223.5- 255.5)	(+13.8)- (-26.3)	(-86.6)- (-101.0)	(-5.1)- (-9.4)	(-13.8)- (-20.4)
With citric acid								
4th-6th	53.0	37.7	76.1	107.0	-25.8	-38.2	-4.1	-5.2
7th-9th	61.7	32.8	61.0	111.1	-17.2	-36.5	-3.0	-4.9
10th-12th	51.8	29.8	57.2	91.6	-3.5	-14.2	-2.9	-3.1
Total:	166.5	100.3	194.3	309.7	-46.5	-88.9	-10.0	-13.2
9 days	(131.2- 213.4)	(61.3- 123.5)	(149.7- 217.8)	(292.3- 345.7)	(-20.9)- (-68.1)	(-31.6)- (-124.9)	(-4.2)- (-14.3)	(-10.5)- (-18.8)

Table 22. Effect of Citric Acid and Vitamin D on Growth, Bone Ash and Serum Ca and P (0480)

Vitamin D	Body weight In- ital	Femur ash	Femur ash			Metaphyseal widths	Serum Ca	Serum inorg. P
			Initial ^b	Final	Loss			
Equalized intake ^a								
Without citric acid	0 +	80 79	16 6	45.4 51.2	-- --	52.6 65.3	-- --	12.6 (2) 15.8 (2)
With citric acid	0 +	76 77	18 11	45.7 48.3	-- --	52.9 58.3	-- --	12.0 (5) 11.6 (4)
<i>Ad libitum</i> intake ^c								
Without citric acid	0 +	77 79	40 11	29.4 43.7	58.5 60.0	42.1 57.3	16.4 2.7	1.6 0.2 11.9 (5) 15.2 (3)
With citric acid	0 +	77 77	38 33	28.3 35.8	58.5 58.5	38.6 48.3	19.9 10.2	1.8 0.4 11.9 (5) 15.9 (6)

^a Average values obtained on the 12th day from six to seven rats in each group except when designated by lesser numbers enclosed in parentheses.

^b Average value from five rats for each group as received from the breeders.

^c Average values obtained on the 21st day from six rats in each group.

Table 23. The Effect of Citric Acid on Insulin-induced Rumplessness (White Leghorn Embryos) (1309)

All injections at 24 hours
Dosage: insulin 2 units; citric acid 25 mg

Dosage: insulin 2 units, citric acid 25 mg

TREATMENT	FERTILE EGGS	MORTALITY FIRST 6 DAYS	SURVIVORS OF 17TH DAY	RUMPLESS (ALL TYPES)	OTHER ANOMALIES
Insulin	165	52.1	65	29.2	9.3
Insulin + citric acid	171	17.0	135	11.1	2.9
Citric acid	165	3.6	157	1.3	0

2. In 1968 Beaudoin (0174) investigated whether citric acid would modify the teratogenic effects of trypan blue in the chicken eggs. Fertile eggs of White Leghorn chickens were injected into the yolk sac at 36 hours incubation with first 25 mg citric acid in 0.1 ml saline followed immediately by 0.1 ml of a 0.1% saline solution of trypan blue. Control eggs were either injected with saline or incubated without injection. The embryos were recovered and examined on the tenth day of incubation. The results are summarized in Table 24. They show that it is possible to interfere with the teratogenic action of trypan blue in the chicken egg by simultaneous treatment with citric acid. These results confirmed those of Landauer (see this section C1 above).

Table 24. Effect of Citric Acid and Trypan Blue on Chick Development When Injected into Eggs of Three Different Flocks at the 36th Hour of Incubation¹ (0174)

	Total Treated	Percentage Mortality	Percentage Malformed Survivors	Percentage All Eggs Affected
Flock 1				
Untreated controls	231	5.6	2.3	7.8
Saline controls	135	17.0	5.4	21.4
Citric acid	182	17.0	1.3	18.1
Trypan blue	226	41.2	30.3	58.8
Trypan blue & citric acid	462	27.3 (P = 0.001) ²	10.1 (P = 0.001)	34.6 (P = 0.001)
Flock 2				
Untreated controls	134	11.9	3.4	14.9
Saline controls	141	12.0	2.4	14.2
Citric acid	77	5.2	1.4	6.5
Trypan blue	94	56.5	56.2	71.2
Trypan blue & citric acid	153	29.8 (P = 0.001)	19.3 (P = 0.001)	43.5 (P = 0.001)
Flock 3				
Untreated controls	117	2.6	0.8	3.4
Saline controls	121	11.5	1.9	13.2
Citric acid	91	8.8	6.0	14.3
Trypan blue	156	49.4	45.6	72.4
Trypan blue & citric acid	134	61.9	31.4 (P = 0.10)	73.9

¹ Dosage: 0.1 mg trypan blue; 25 mg citric acid.

² Probability determined by chi-square method.

BIOCHEMICAL ASPECTS

I. Breakdown

Clements (0439) found definite changes in citric acid levels of two varieties of oranges during maturation and senescence (see Table 25).

Table 25. Seasonal Changes in Citric Acid Concentration of Oranges (0439)

Variety	Season	Citric Acid Concentration (meq/fruit)
Valencia-pulp	November	26
	March (early maturity)	<20
	December	9
Navel-pulp	February (after maturity)	16
	July	10

Sweeney *et al.* (2302) reported that the citric acid concentration in potatoes during storage tended to increase and that the change was greater at 70°F than at 55°F (see Table 26). The authors pointed out that their results differed somewhat from those of Minina and of Schwartz who found an initial decrease in the citric acid content followed by an increase. The latter investigator, however, employed storage temperatures of 38°F followed by 45 and 55°F (references given in original article).

Dostal *et al.* (0564) studied changes in the non-volatile organic acids of broccoli treated after harvest with N⁶-benzyladenine in comparison with untreated control samples over a 5-6 day period at 25°C (N⁶-benzyladenine is a phytokinin reputed to "extend storage life of some commodities and enhance their market acceptability").

The results presented in Figure 5 show marked changes in the citric acid levels of both treated and control samples. Probable mechanisms involved in these changes in relation to protein and amino acid breakdown and the Krebs cycle (Figure 6) are discussed.

Table 26. Acid Content of Potatoes as Affected by Storage Time and Temperature¹ (2302)

Year and section	Storage time, months	Storage Temp., °F	Titratable acids %			Malic acid mg/100 g			Citric acid mg/100 g			Ascorbic acid mg/100 g			Pyroglutamic acid mg/100 g		
			Pungo	Rosemont Cobbler	Russet Burbank	Pungo	Rosemont Cobbler	Russet Burbank	Pungo	Rosemont Cobbler	Russet Burbank	Pungo	Rosemont Cobbler	Russet Burbank	Pungo ²	Rosemont Cobbler	Russet Burbank
1965																	
Stem	0	...	0.104b	0.066ab	0.081a	91b	40a	75a	174b	116a	224a	22.2a	21.2a	18.3a	...	4	4
	1	70	0.122a	0.078a	0.071a	110ab	34a	57ab	258a	150a	224a	16.5b	15.5b	14.8b	...	6	8
	2	55	0.126a	0.069ab	0.080a	120a	31ab	62a	204b	96a	195a	13.6b	13.4b	13.7b	12	4	4
	5	55	0.085c	0.072ab	0.075a	75b	31ab	54b	247a	104a	197a	8.5c	9.2c	10.8c	...	3	10
Bud	0	...	0.128b	0.085ab	0.103a	114ab	60a	74a	237ab	169a	324a	25.6a	25.5a	22.9a	...	4	5
	1	70	0.154ab	0.099a	0.103a	117ab	42ab	67a	341a	220a	370a	17.9b	17.6b	18.0b	...	4	12
	2	55	0.159a	0.101a	0.111a	139a	57a	75a	253ab	183a	340a	14.2b	15.4b	16.2b	18	2	6
	5	55	0.114b	0.094ab	0.100a	112b	51a	59ab	329a	190a	329a	10.0c	12.0c	11.5c	...	9	16
1966																	
Stem	0	...	Pungo	Irish Cobbler	Pungo	Irish Cobbler	Pungo	Irish Cobbler	Pungo	Irish Cobbler	Pungo	Pungo Cobbler	Pungo	Irish Cobbler	...	34	
	1	70	0.120a	0.088a	50ab	82a	274c	340ab	34.1a	24.8a	34		
			0.112ab	0.089a	34b	68a	355b	375ab	24.8b	18.4b	53		
	2	55	0.092ab	0.092a	52ab	68a	313c	380a	21.0b	13.1c	55		
	5	55	0.122a	0.084a	97a	71a	487a	381a	12.6c	10.8d	5		
Bud	0	...	0.150a	0.098a	69bc	100a	407b	412ab	47.6a	29.4a	6		
	1	70	0.139ab	0.122a	50c	106a	438b	460a	31.2b	22.6b	9		
	2	55	0.126ab	0.103a	91b	100a	433b	454a	24.3b	16.4c	59		
	5	55	0.152a	0.100a	138a	98a	639a	483a	14.1c	13.2d	6		

¹a differs from b at 1% level, a differs from ab at 5% level.².... indicates no detectable amount.

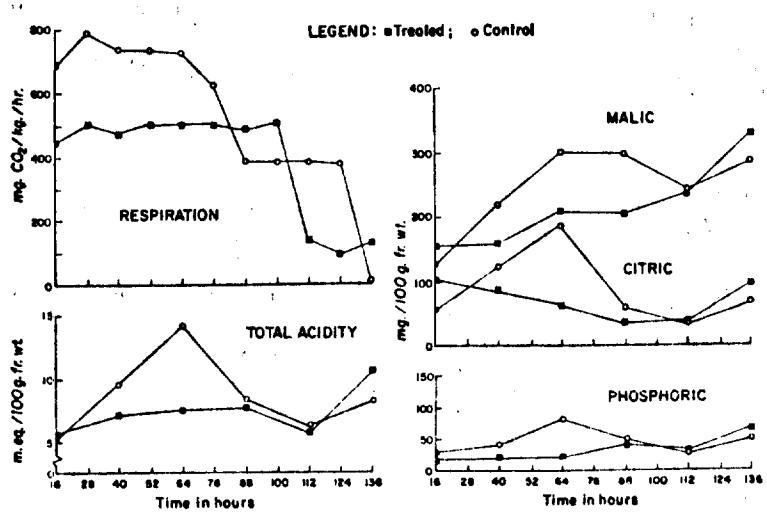


Figure 5. The Time Course Changes in Respiration Rates and Various Acid Fractions in Broccoli Treated with N^6 -benzyladenine and Corresponding Control Samples (0564)

Packer *et al.* (1755) stated that when citric acid is heated alone to about 150°C , dehydration occurs and aconitic acid ($\text{CH}\cdot\text{COOH} = \text{C}\cdot\text{COOH}-\text{CH}_2\cdot\text{COOH}$) is formed. Lockwood *et al.* (1389) indicated that this change takes place at 175°C and that aconitic acid loses CO_2 to form itaconic anhydride which in turn may add water to form itaconic acid ($\text{CH}_2 = \text{C}\cdot\text{COOH}-\text{CH}_2\cdot\text{COOH}$) or rearrange to form citraconic anhydride which gives citraconic acid ($\text{CH}_3-\text{C}\cdot\text{COOH} = \text{CH}\cdot\text{COOH}$) on addition of water. The authors report also that when citric acid is heated in air or with an oxidizing agent (details not given), acetonedicarboxylic acid ($\text{COOHCH}_2\text{COCH}_2\text{COOH}$) is formed which may decompose to carbon dioxide and acetone (1389). However, neither group of investigators indicated whether conditions which bring about changes such as those reported obtain in any area of the food industry or in the home.

Dilute aqueous solutions of citric acid are susceptible to attack by certain micro-organisms and may ferment on standing (2247).

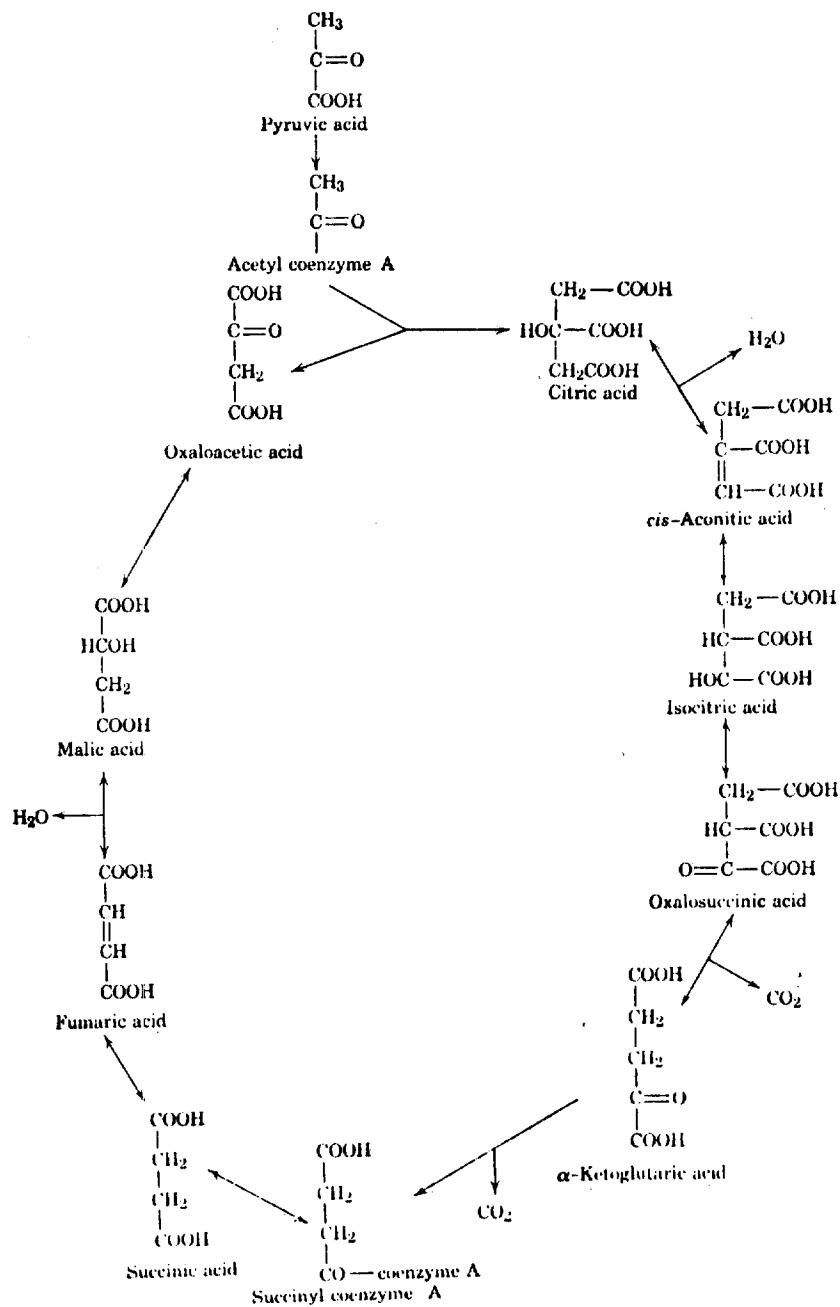


Figure 6. Krebs (citric acid) Cycle (0564)

II. Absorption and Distribution

	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
A. Absorption	Rats	Citric acid was absorbed, as it did not appear in the feces and was not destroyed by intestinal contents. The rat could metabolize maximum non-fatal quantities.	1940	1278
	Rats	Sucklings (10 g) intubated with citric acid 400 mg/kg absorbed and metabolized it more slowly than adults. Conclusion: possibly relevant to problems with acidulated infant formulas reported by some authors but denied by others.	1966	0182
	Humans	Intubation studies indicated citric acid had little influence on the rate of gastric emptying.	1962	1017
B. Distribution	<u>In vitro</u>	Present in normal fibrin.	1960	0111
	<u>In vitro</u>	Rabbit liver mitochondria strongly accumulated citrate.	1965	0736
	Mice	Less citrate than acetate was incorporated into fatty acids of carcass and liver; most citrate appeared in stearic acid. Less citrate than acetate appeared in liver mitochondria, but equal amounts in microsomes and cytosol.	1970	0094

III. Metabolism and Excretion

	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
A. Metabolism	Humans	Free citric acid was metabolized differently from that in food. In infants, free citric acid did not alter the citrate level of gut contents, but lowered the urinary output of citrate from food. The authors stated that this was comparable to adults and other mammals.	1940	2201
	Humans	Citrate tolerance tests during surgery did not change, while glucose and lactate tolerance did. The authors concluded that citrate became an energy substrate during surgery.	1961	0571
	Humans	Citrate absorption was not altered during surgery. Surgery did not impair the Krebs cycle, though glycolysis was blocked at two steps. Citrate was recommended as an energy source during surgery.	1962	0572
	Human tissue <u>in vitro</u>	Plasma-free platelets: the Krebs cycle produced ATP when citrate was the substrate. Glucose produced lactate.	1967	1120
	<u>In vitro</u>	Citrate metabolism, illustrated in Figures 7 and 8, suggested that ATP level was balanced for energy-yielding and energy-requiring reactions by the adenylate system and the Krebs cycle. The cycle supplied electrons for electron-transport phosphorylation. The citrate-isocitrate step was important.	1968	0113

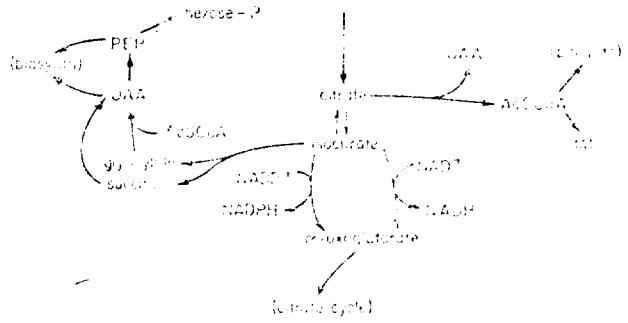


Figure 7. Schematic Representation of Major Pathways of Citrate Metabolism (0113)

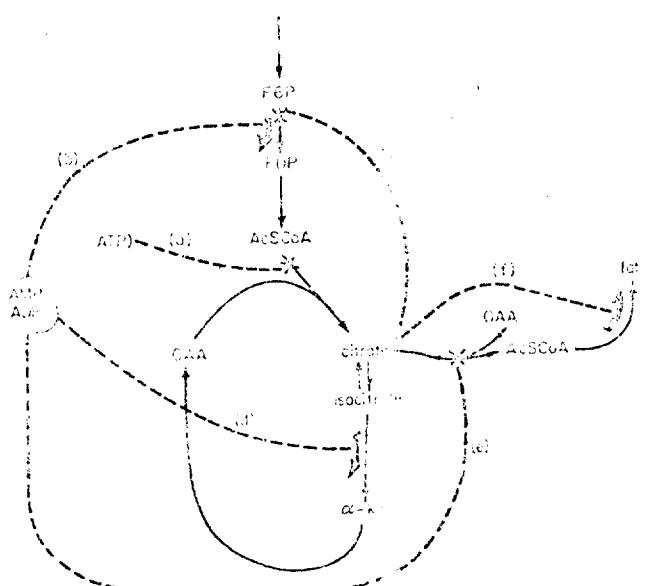


Figure 8. Some Regulatory Interactions Affecting the Production and Utilization of Citrate. Broken Lines Link Modifiers to the Reactions that They Control. Negative Modifier Action is Indicated by a Cross, and Positive Modifier Action by an Arrow. (0113)

III. Metabolism and Excretion (Cont'd)

	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
	Rats	1 or 5 g/kg p.o. Increased Krebs cycle metabolism, inhibited glycolysis, ketone production; stimulated lipogenesis, gluconeogenesis, urea production.	1969	0153
B. Excretion	Dogs, Humans	Citric acid always found in urine. Amount always varied with pH. Sodium bicarbonate increased urinary output of citric acid.	1936	2136
	Humans	Urinary output of citric acid was higher in normal adults than in children or the aged. Those with calcified stones retained more citric acid, had normal blood levels, and lower urine levels, than those without stones, and citrate in kidney or urine was more efficiently oxidized.	1943	2107
	Humans	In 116 healthy infants, in urine, 6-8 ml/min/1.73m ² body surface, of which 92% was reabsorbed by the tubules.	1968	2398

IV. Effects on Enzymes and Other Biochemical Parameters

<u>Parameter</u>	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
Trypsin	<u>In vitro</u>	Presence of citrate in fibrin enhanced activity of trypsin and other plasminogen activators.	1960	0111
Phosphofructo-kinase	Rat heart muscle	Citrate inhibits PFK, and this inhibits hexokinase. Thus citrate controls glycolysis.	1969	1905
Phosphorus	Dogs	Citrate 57 ng/kg i.v. lowered blood P by 30%, low point 2 hours after administration.	1963	1316
Vitamin C	Humans	Adults: 5mg/day p.o. for 4 days diminished the utilization of vitamin C and caused C depletion. Citrate contributes to production of oxalate.	1968	2626
Vitamin D & calcium		See monograph on Vitamin D.		
Copper	Human serum <u>in vitro</u>	Citrate inhibits ceruloplasmin.	1964	1734
Coagulation	Human platelets <u>in vitro</u>	Citrate acts as anticoagulant.	1965	0110
Marrow	Rabbit <u>in vitro</u>	Citrate slightly increased O ₂ consumption, accelerated glucose consumption, increased the production of lactic and pyruvic acids.	1956	1024
Uric acid	Humans	10 g/day p.o. of citric acid and salts caused patients with urate stones to excrete more citrate than normals; blood urate decreased more in normals than in patients with stones.	1968	1504

IV. Effects on Enzymes and Other Biochemical Parameters (Cont'd)

<u>Parameter</u>	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
Cholesterol	Rabbits	960 mg/kg of citrate accelerated lowering of cholesterol in blood, liver, adrenals, and aorta after stopping cholesterol feeding. Some aortic esterases returned to normal activities.	1968	1105
Glycogen	Rats	Citrate was as effective as glucose at relieving insulin hypoglycemia; it increased the liver glycogen levels.	1940	1425
Taste-sense	Humans	Sensory evaluation: citric acid and sucrose masked each other; citric acid increased the salty taste of NaCl.	1964	1767
‡ Taste-sense	Humans	Sourness of citrate was reduced by sucrose or NaCl. Citrate reduced sweetness of sucrose; NaCl enhanced it. Saltiness of NaCl was reduced by sucrose, increased by citrate.	1964	1768

V. Drug Interactions

<u>Drug</u>	<u>Species</u>	<u>Findings and Conclusions</u>	<u>Year</u>	<u>Reference</u>
KCl	Cows	Citric acid with KCl lowers blood Mg faster than does KCl alone. KCl plus citrate produced tetany, but citrate alone did not. All treatment was p.o.	1969	0253

VI. Consumer Exposure Information

Citric acid has been used widely in the food industry for more than 100 years as an acidulant, sequestering agent, synergistic antioxidant, dispersing agent, flavor enhancer, and water conditioning agent (0724,0930, 2247). Its great solubility, pleasant sour taste, very low toxicity, and low cost have made it the preferred acidulant in food processing (1389). Moreover, it is completely consumed in the animal body, according to Thunberg (2392), with a combustion value of 2.47 Cal/gram. Faith (0647) points out, however, that the cheaper fumaric acid is rapidly becoming competitive and may displace citric acid to a certain extent in the food industry.

Citric acid is classified GRAS as a miscellaneous and/or general purpose food additive as indicated above (0724). It is approved also under USDA meat inspection regulations as indicated in Table 34.

A. NAS/NRC Survey Data

The estimated amounts of citric acid in average daily diets as reported in the Comprehensive GRAS Survey and given in Table 27. The estimated maximum amount in the daily diet is 3792.533808 mg for infants and children up to 2 years of age and 6792.227180 mg for the age group 2 to 65+ years (0678). Citric acid is used at the maximum level in reconstituted vegetables (R), 3.3%, wtd. mean (0679). The annual poundage data are given in Table 28. See Tables 29, 30, 31, 32, 33, and 34 for other data.

B. FAO/WHO Acceptable Daily Intake (ADI) Estimates

The Joint FAO/WHO Expert Committee on Food Additives (1086), in 1962, gave the following estimated Acceptable Daily Intake zones for man: unconditional, 0-60 mg/kg BW; conditional, 60-120 mg/kg BW (amounts occurring naturally not included).

C. Domestic Production and Import Poundage

Actual citric acid production data are not available but domestic production estimates range from 60 to 80 million pounds annually. Almost all is produced by fermentation although a small amount is obtained from pineapple canning wastes or from orange or lemon waste products (1389).

In 1970, imports from abroad totaled 260,155 pounds (2443). In January and in February of this year (1974), imports were 40,000 pounds (2444).

Table 27. Estimated Amount of Citric Acid in the Average Daily Diet (0678)

Age Groups	No. of Firms	Possible Average Daily Intake, mg
0-5 mo.		611.525360
6-11 mo.		1160.531920
12-23 mo.	247	1795.061160
2-65+ yrs.		3124.698814

D. Research Reports

The main use of citric acid in the food industry is as an acidulant and flavor enhancer for soft drinks, instant beverage powders, fruit juices, hard candies and gelatin desserts (0647).

Mitchell *et al.* (1573) were granted a U. S. patent in 1962 for effervescent beverage powders containing as much as 56.7% citric acid together with a metal carbonate or bicarbonate, sweetener, flavor and color ingredients. The material was packaged in small aluminum foil envelopes for reconstitution in 200 ml of water to give a 7 ounce instant carbonated beverage drink.

Gonce *et al.* (0802) developed a citric acid milk recommended for infant feeding under certain conditions on the basis of improved curd characteristics and better digestibility. The acidulated product is prepared by the addition of 4 grams of anhydrous citric acid per quart of milk.

Citric acid together with diacetyl, starter distillate, and other flavoring substances was approved recently (1967) for use in the preparation of creamed cottage cheese (0083).

Murthy *et al.* (1629), in 1961, developed a method for removing radioactive Sr^{85} , Ba^{140} , or Cs^{134} from market milk by acidification with citric acid (0.1 M or 0.5 M) to pH 5.4 or 5.3 and passage through a column of Dowex 50W-X8 cationic resin in the CaMgKNa cycle, followed by mixing with Dowex 2-X8 anionic resin in the OH^- cycle with readjustment to pH 6.6. Spent resin was removed by filtration through cheesecloth. Approximately 90-95% of Sr^{85} , 85-95% of Ba^{140} , and 75% of Cs^{134} were removed. Further treatment removed 30 to 90% of the remaining radio-nuclides.

Table 28. Annual poundage data for NAS Appendix A substances (Groups I and II)
 (Comprehensive GRAS Survey, NAS/NRC 1972, Table 11, Part A) (0680)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS)		TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA 1970 ONLY	POUNDAGE REPORTED TO FEMA 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
		1960	1970				
CITRIC ACID NAS 0068	116/137 FEMA 2306	22,758,950	24,076,522	27,162,041	109	8,736,420	35,898,461

Table 29. Possible daily intakes of NAS Appendix A substances (Groups I and II), per food category and total dietary, based on food consumption by total sample (Comprehensive GRAS Survey, NAS/NRC 1972, Table 13, Part A) (0678)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	***** POSSIBLE DAILY INTAKE, MC. *****			
			AGE	AVERAGE	HIGH A	HIGH S
CITRIC ACID NAS 0068	01 BAKED GOODS(R)	43	0-5 MO.	.7229740	.9567450	.9.007950
			6-11 MO.	.54.002540	110.131980	67.294760
			12-23 MO.	115.872450	190.923780	144.392300
			2-65+ YR.	291.700920	433.299180	363.497680
CITRIC ACID NAS 0068	02 BREAK CERLS(R)	4	0-5 MO.	.076030	.215560	.676080
			6-11 MO.	2.827640	7.382640	2.827640
			12-23 MO.	3.303400	6.454120	3.303400
			2-65+ YR.	2.534600	6.560240	2.536000
CITRIC ACID NAS 0068	03 OTHER GRAIN(R)	7	0-5 MO.	.199600	.678640	.199700
			6-11 MO.	3.872240	11.417120	3.874180
			12-23 MO.	6.544880	15.129680	6.550160
			2-65+ YR.	11.097760	24.510380	11.103320
CITRIC ACID NAS 0068	04 FATS OILS(R)	22	0-5 MO.	.231900	.231900	.418450
			6-11 MO.	1.298640	3.478500	2.343320
			12-23 MO.	2.921540	5.565600	5.272470
			2-65+ YR.	8.116500	14.656080	14.645750
CITRIC ACID NAS 0068	05 MILK PRODS(R)	7	0-5 MO.	4.456620	3.301200	7.251120
			6-11 MO.	51.496720	247.672530	83.790720
			12-23 MO.	44.978950	143.932320	73.182600
			2-65+ YR.	32.599350	99.531180	53.040600
CITRIC ACID NAS 0068	06 CHEESE(R)	6	0-5 MO.	*****	.030430	*****
			6-11 MO.	1.037610	3.727710	2.346400
			12-23 MO.	2.997540	8.531460	6.774760
			2-65+ YR.	3.612420	9.069480	8.170430

Table 29 (Cont'd)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	***** (AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	***** HIGH B
CITRIC ACID NAS 0068	07 FRCZN DAIRY(R)	38	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	1.309200 12.437400 16.852480 33.515520	5.367720 34.562880 44.250960 80.777640	2.910600 27.650700 41.912640 74.511360
CITRIC ACID NAS 0068	08 PROCSE FRUIT(R)	27	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	60.640810 668.339140 1297.971360 1526.342090	162.568930 1664.396700 2576.589310 3233.316380	74.855940 825.008240 1662.236080 1084.140440
CITRIC ACID NAS 0068	09 FRUIT ICES(R)	10	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.000000 .302310 .604620 .705390	.000000 .806160 2.116170 2.510250	.000000 .545910 1.091820 1.273790
CITRIC ACID NAS 0068	10 MEAT PROCS(R)	17	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.896610 16.872870 24.615020 63.903840	2.363790 45.482580 42.303690 106.044510	.976360 18.373320 26.805520 69.587040
CITRIC ACID NAS 0068	11 POULTRY(R)	*	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.485500 3.821220 6.466680 12.635420	2.253540 12.933360 18.028320 32.137440	.489900 3.821220 6.466680 12.639420
CITRIC ACID NAS 0068	12 EGG PROCS(R)	*	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** .300000 .340000 1.140000	.600000 1.080000 2.340000 4.140000	***** .300000 .540000 1.140000
CITRIC ACID NAS 0068	13 FISH PROCS(R)	6	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.630970 .402610 1.672380 3.842280	.092910 1.517530 4.180950 9.569720	.039380 .511940 2.126520 4.803120
CITRIC ACID NAS 0068	14 PROCSE VECS(R)	23	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.654580 11.909100 19.351800 42.177000	2.034040 27.787200 32.401860 71.055840	1.611600 27.626800 44.896800 97.852000
CITRIC ACID NAS 0068	15 CONDM RELSH(R)	11	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** .300480 1.051680 3.305200	.037560 .823320 2.854560 7.962720	***** .645120 2.257920 7.096320

Table 29 (Cont'd)

SUBSTANCE NAME (SURVEY NO.)	FCCD CATEGORY NO. NAME	# OF FIRMS	***** (AGE)	AVERAGE	POSSIBLE DAILY INTAKE, MG. HIGH A	***** HIGH B
CITRIC ACID NAS 0068	16 SOFT CANDY(R)	44	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.437140 4.828540 7.649950 12.677060	4.371400 14.826760 20.327010 38.468320	2.751580 30.267380 48.152650 79.799320
CITRIC ACID NAS 0068	17 CONE FROST(R)	6	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** .227690 .055380 .083070	.227690 .055380 .193830 .221520	***** .092500 .185000 .277500
CITRIC ACID NAS 0068	18 JAM JELLY(R)	19	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** 15.200960 6.806400 12.932160	.680640 50.594240 25.410560 40.157760	***** 36.910970 16.527300 31.401870
CITRIC ACID NAS 0068	19 SWEET SAUCE(R)	11	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.797730 2.393190 6.913660 18.081880	1.063640 8.243210 20.209160 47.597890	.991440 2.974320 8.592480 22.472640
CITRIC ACID NAS 0068	20 GELATIN PUD(R)	33	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	5.245400 33.570560 36.193260 53.503060	7.081290 101.760760 88.122720 137.351750	7.783400 49.016320 58.700220 79.394760
CITRIC ACID NAS 0068	21 SOUPS(R)	7	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	.039020 4.545830 6.785440 6.184670	.292650 14.183770 18.749110 16.485970	.044460 5.179590 7.738040 7.044610
CITRIC ACID NAS 0068	22 SNACK FOODS(R)	8	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** .150760 .414590 .489970	.037690 .414590 1.162390 1.394530	***** .433200 1.151520 1.408160
CITRIC ACID NAS 0068	23 REV TYPE I(R)	69	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	4.007520 38.755710 52.535660 177.559200	6.146280 132.657210 277.436250 474.117210	7.067760 66.349230 159.613560 306.269400
CITRIC ACID NAS 0068	24 REV TYPE II(R)	15	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YR.	***** ***** ***** 215.104500	.000000 .661360 1.323720 624.795840	.000000 ***** ***** 268.264750

Table 29)Cont'd)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG.		HIGH A HIGH B
			(AGE)	AVERAGE	
CITRIC ACID NAS 0068	25 NUT PRCDS(R)	*	0-5 MO.	*****	.000220
			6-11 MO.	.004070	.014740
			12-23 MO.	.002970	.009900
			2-65+ YR.	.005720	.017050
CITRIC ACID NAS 0068	26 RECENT VEG(R)	*	0-5 MO.	.000000	.000000
			6-11 MO.	.000000	.000000
			12-23 MO.	.000000	.000000
			2-65+ YR.	6.600000	19.800000
CITRIC ACID NAS 0068	27 GRAVIES(R)	12	0-5 MO.	.099240	.297720
			6-11 MO.	1.389360	3.870360
			12-23 MO.	3.572640	10.122480
			2-65+ YR.	8.236920	21.138120
CITRIC ACID NAS 0068	28 IMIT DAIRY(R)	4	0-5 MO.	.000000	.000000
			6-11 MO.	2.930480	4.914300
			12-23 MO.	1.674560	7.116880
			2-65+ YR.	1.883880	3.139000
CITRIC ACID NAS 0068	30 HARD CANDY(R)	21	0-5 MO.	.000000	.000000
			6-11 MO.	.554680	1.664040
			12-23 MO.	1.664040	4.992120
			2-65+ YR.	3.326000	9.429560
CITRIC ACID NAS 0068	31 CHEWING GUM(R)	*	0-5 MO.	*****	*****
			6-11 MO.	.783490	.783490
			12-23 MO.	.783490	2.350470
			2-65+ YR.	1.566980	3.133960
CITRIC ACID NAS 0068	34 INS CCF TEA(R)	*	0-5 MO.	.940000	15.510000
			6-11 MO.	24.910000	61.570000
			12-23 MO.	29.140000	105.280000
			2-65+ YR.	569.170000	1219.180000
CITRIC ACID NAS 0068	48 SEAS FLAVRS(R)	7	0-5 MO.	*****	*****
			6-11 MO.	*****	.059874
			12-23 MO.	*****	.119748
			2-65+ YR.	.059874	.299370
CITRIC ACID NAS 0068	82 CEREALS(B)	*	0-5 MO.	58.050000	100.190000
			6-11 MO.	39.560000	103.630000
			12-23 MO.	8.600000	36.700000

Table 29 (Cont'd)

SUBSTANCE NAME (SURVEY NO.)	ECOD. CATEGORY NO. NAME	# OF FIRMS	***** (AGE)	POSSIBLE DAILY INTAKE, MG.	***** HIGH A	***** HIGH B
CITRIC ACID NAS 0068	83 FORMULAS(B)	*	0-5 MO. 6-11 MO. 12-23 MO.	402.638680 824.838930 28.586600	737.631000 390.666460 7.436200	402.638580 82.038940 22.386900
CITRIC ACID NAS 0068	84 PRECSE FRUT(B)	*	0-5 MO. 6-11 MO. 12-23 MO.	52.773600 54.212680 13.433220	125.667120 170.714600 56.211880	78.889800 81.041340 20.081040
CITRIC ACID NAS 0068	90 PUDDINGS(B)	*	0-5 MO. 6-11 MO. 12-23 MO.	6.081420 7.437010 2.371460	22.247220 27.695870 2.331380	14.533630 17.845000 4.667190
CITRIC ACID NAS 0068	92 MEAT DINNER(B)	*	0-5 MO. 6-11 MO. 12-23 MO.	1.770600 12.925380 1.062360	7.002460 51.347400 3.364140	1.770600 12.925380 1.062360
CITRIC ACID NAS 0068	93 COND. CINER(B)	*	0-5 MO. 6-11 MO. 12-23 MO.	2.200000 5.080000 1.560000	7.940000 14.220000 5.900000	2.300100 5.010000 1.550010
CITRIC ACID NAS 0068	ALL CAT(C SERIES)	247	0-5 MO. 6-11 MO. 12-23 MO. 2-65+ YRS.	611.526260 1160.031520 1795.861160 3124.698014	1225.598680 3321.050134 3792.533958 6792.227150	681.221420 1536.042270 2373.359170 4045.700424

Table 30. Usage levels reported for NAS Appendix A substances (Group I) used in regular foods (R)
 (Comprehensive GRAS Survey, NAS/NRC 1972, Table 2) (0679)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CITRIC ACID NAS 0068 FEMA 2306	01. BAKING POWD(R)	43	.21261	.26424
	02. BREAK CERED(R)	4	.01268	.01268
	03. OTHER GRAINS(R)	7	.03992	.03994
	04. FATS OILS(R)	22	.04638	.08369
	05. MILK PRODS(R)	7	.09253	.13428
	06. CHEESE(R)	5	.03843	.08692
	07. SOFT DAIRY(R)	38	.13692	.29106
	08. BAKED POUT(R)	27	1.29623	1.59760
	09. FRUIT JUGGL(R)	10	.16177	.16177
	10. NUT PRODS(R)	17	.01651	.00173
	11. ICE CREAM	8	.09714	.09714
	12. EGG PRODUCTS	4	.06940	.06940
	13. FISH FOODS(R)	6	.03097	.03097
	14. PROCSD VEGS(R)	23	.04962	.11512
	15. CONCD RELISH(R)	11	.03756	.08064
	16. SOFT CANDY(R)	44	.21857	1.37579
	17. CONF PASTRY	6	.02749	.00250
	18. JEL JELLY(R)	19	.22638	.55091
	19. SWEET SAUCE(R)	1	.26591	.33048
	20. GELATIN PUDIR(R)	33	.26227	.36919
	21. SOUPS(R)	7	.01951	.02223
	22. SNACK FOODS(R)	8	.03769	.10832
	23. BEV TYPE I(R)	69	.17073	.29449
	24. BEV TYPE II(R)	15	.66185	.82543
	25. NUT PRODS(R)	*	.00011	.00011
	26. RECOND VEG(R)	*	3.30000	3.30000
	27. GRAVIES(R)	12	.09924	.16258
	28. IMIT DAIRY(R)	4	.20932	.20946
	30. HARO CANDY(R)	21	.55468	2.26507
	31. CHEWING GUM(R)	*	.78349	1.05750
	34. INS CEF TEAR(R)	*	.47000	.50000
	48. SEAS FLAVRS(R)	7	.59874	.59874
CITRIC ACID NAS 0068 FEMA 2306	(CONT.) 49. MISCELLANEOUS(R)	*	.13368	.26744

Table 31. Usage levels reported for NAS Appendix A substances (Group II) used in infant formula products and baby foods (B) (Comprehensive GRAS Survey, NAS/NRC 1972, Table 3) (0677)

NAS SURVEY NO. SUBSTANCE NAME	FOOD CATEGORY NO. NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0012 CITRIC ACID	82. CEREALS(B)	*	.43000	.47000
	83. FORMULAS(B)	*	.11994	.11994
	84. PROCSD FRUT(B)	*	.07996	.11903
	85. SOUP(LIQUID)(I)	*	.07600	.19307
	87. SOFT LIPSTICK(B)	*	.17700	.17700
	93. CONF DUMPLING(B)	*	.01000	.01000

Table 32. Citric Acid Usage Levels in Foods (0681)

Foods in Which Used	Approx. Avg. Maximum ppm
Beverages	2,500
Ice Cream, Ices	1,600
Candy	4,300
Chewing Gum	3,600

Table 33. Some Applications of Citric Acid in Food Processing (0681)

Lard	0.001 - 0.01%
Frozen peaches	
Grape wine	
Canned fish cakes	0.05%
Pie-crust mix	0.05%
Prepared breakfast cereal	0.002%
Soup base	0.0002%
Antioxidant salt	0.035%
Used to assist dispersion of finings in brewing industry.	
Up to	0.005%
Oleomargarine	
Rendered animal fat or a combination of such fat and vegetable fat. Up to	0.01%
Neutralizer after lye peeling	
Adjustment of pH of fruit juices, wines, jams, jellies, jelly candies, canned fruit, carbonated beverages, frozen fruit, canned vegetables, frozen dairy products	
Cheese spread	0.75%
Sherbet	0.32%
Confectionery. Not more than	4.0%
Canned figs	
Dried egg whites	
Cheese products. Same as for Acetic Acid	
Mayonnaise, salad dressing, french dressing. May be mixed with vinegar in such quantity that the weight of the citric acid is not greater than 25% of the weight of acids of the vinegar calculated as acetic acid	
Fruit butter, jelly, preserves, jams. May be added in amount sufficient to compensate for deficiency of fruit acidity	
Canned vegetables. Citric acid or a vinegar, in the cases of all vegetables (except artichokes, in which such ingredient is prescribed, and except canned mushrooms, in which no such ingredient is permitted), in a quantity not more than sufficient to permit effective processing by heat without discoloration or other impairment. Artichokes—citric acid or vinegar is added in such quantity as to reduce the pH of the finished canned vegetable to 4.5 or below	
Fresh beef blood. Citric acid or sodium citrate with or without water, may be added to blood in an amount up to 0.2% of the total mixture	
Cultured buttermilk	0.12 - 0.2%

Table 34. Approval and Restriction on Use of Citric Acid in Meat and Poultry Products (0085)

<u>Product</u>	<u>Purpose</u>	<u>Level Used</u>
Chili con carne	Flavoring agent	Sufficient for the purpose
Oleomargarine	Protect flavor	Do
Poultry products	Do	Do
Lard & shortening	Synergist to increase effectiveness of antioxidants	0.01%
Poultry fats	Do	0.01%
Dry sausage	Do	0.003%
Cured poultry products	Curing agent to accelerate color fixing or preserve color during storage	See below ^a
Cured pork & beef cuts	Do	Do
Cured comminuted meat food products	Do	Do

^aSufficient to replace up to 50% of ascorbic acid, erythorbic acid, sodium ascorbate, or sodium erythorbate used.

Permission was granted in 1970 for the use of a lemon flavoring (lemon oil and citric acid) at a level of 0.005% by weight in canned tuna (0084).

Citric acid, at a level of 0.01%, is permitted as a synergist with antioxidants butylated hydroxyanisole, propyl gallate, and non-dihydroguaiaretic acid in fresh pork sausage and freeze dried meats to retard rancidity (0082).

Lineweaver *et al.* (1381) found that Tenox II (20% butylated hydroxyanisole, 6% propyl gallate, 4% citric acid, and 70% propylene glycol) at a level 0.005% by weight, was markedly effective in retarding development of rancid flavors and peroxide during cooking and frozen storage of creamed turkey.

Nebesky *et al.* (1654) found that citric acid at levels of 0.25% or 0.5% (by wt.) effectively stabilized the color of strawberry fountain syrup during storage.

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